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## Phytochemical profiling, *in-vitro* antioxidant and anti-inflammatory activities of *Hopea ponga*, *Kandelia candel*, *Vitex leucoxydon* and *Rhizophora apiculata*

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### Abstract

Phytochemical investigation and evaluation of *in vitro* antioxidant and anti-inflammatory activities of *H. ponga*, *K. candel*, *V. leucoxydon* and *R. apiculata*. Plant extraction was carried out by soxhlet extraction method with increasing polarity of solvents viz., methanol, ethanol and water. Qualitative phytochemical analysis was performed to investigate the phytochemical content. Antioxidant potential of the plant extracts were analyzed by four different *in vitro* antioxidant assays viz., ferric ion reducing antioxidant power, phosphomolybdenum, hydrogen peroxide scavenging assay and DPPH assay and anti-inflammatory activity was carried out by using protein denaturation *in-vitro* bioassay. Phytochemical analysis of all the four experimental plants revealed that each solvent extracts contained broad spectrum of secondary metabolites including phenolics, flavonoids, tannins and glycosides. Aqueous extracts of *V. leucoxydon* and *R. apiculata* exhibited the highest phenolic content; methanolic extract of *K. candel* exhibited highest flavonoids content and therefore possessed significant antioxidant capacity. *In vitro* anti-inflammatory assay revealed that methanol extract of *K. candel* showed highest anti-inflammatory activity, when compared to all other extracts whereas *K. candel* ethanol extract showed least activity. The present study revealed that different solvent extracts of *H. ponga*, *K. candel*, *V. leucoxydon* and *R. apiculata* leaves contain broad spectrum of bioactive compounds. Results confirm that aqueous extract of *V. leucoxydon* and *R. apiculata* showed significant antioxidant activity and methanol extract of *K. candel* showed high anti-inflammatory activity. Further study requires purification, characterization and structural elucidation of phytochemicals from these extracts that may help in the development of new drug formulations against inflammation.

**Keywords:** Phytochemicals; H<sub>2</sub>O<sub>2</sub> assay; 2,2-diphenyl-1-picrylhydrazyl

### 1. Introduction

Worldwide more than 30,000 plant species have been used for medicinal purposes (Haripriya *et al.*, 2010) [19]. India is a home to thousands of potential medicinal plant species; it is ranked sixth among 12 mega biodiversity countries of the world (Kiruba & Jeeva, 2010; Anpin Raja *et al.*, 2010; Mahesh *et al.*, 2010; Meena *et al.*, 2009; Prakash *et al.*, 2008) [21, 5, 27, 31, 39]. Medicinal plants are backbone of Indian traditional system of medicine. Some of the ethno-medicines have been incorporated in the organized system of medicine, however much larger number of ethno-medicines has remained untouched especially in western ghat region of Karnataka (Bhat satyanarayana, 2004) [7]. Plants are the rich sources of secondary metabolites such as alkaloids, phenols, flavonoids, tannins, saponins, glycosides, terpenoids etc. that possess a wide array of biological properties including antibacterial, antifungal, antioxidant and anticancer (De-Fatima *et al.*, 2006) [14]. Reactive oxygen species (ROS) have been causative agents for many diseases from malaria to hemorrhagic shock to AIDS. Oxidative stress due to free radicals causes various forms of tissue damage and inflammation. Oxidative stress also plays an important role in the development of several degenerative changes in cells and tissues which ultimately leads to several degenerative disorders (Alho & Leinonen, 1999) [4]. Antioxidants are the chemicals which can prevent or slow the oxidative damage of cells. They act as free radical scavengers and hence prevent and repair damage caused due to the free radicals. Recently natural products as antioxidants are gaining high importance due to their high potential in health promotion by preventing the pathogenesis of many diseases including diabetes, cancer and cardiovascular diseases. Plant are rich source of antioxidant phytochemicals, several plants are reported as natural sources for antioxidants viz., phenols, flavonoids etc. Uptake of these antioxidants in dietary supplements will reduce many ailments including the heart diseases (Lekameera *et al.*, 2008; Zadak *et al.*, 2009) [24, 65].

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Inflammation is a prominent phenotype of various diseases such as rheumatoid arthritis, atherosclerosis and asthma, although inflammation is primarily a protective response against pathogens, toxins and allergens (Gil, 2002) [17]. There are many synthetic drugs available to treat inflammation but they have disadvantages because of their detrimental side effects on the gastrointestinal tract, kidneys and on the cardiovascular system and reappearance of symptoms after discontinuation (Srinivasan *et al.*, 2001; Alexandrina, 2010) [55, 3]. Since from ancient times inflammatory disorders and related diseases have been treated with plant or plant derived formulations, because of their specific action and less side effects (Krishnaswamy, 2008; Marc *et al.*, 2008) [23, 29]. In the present study, *Hopea ponga*, *Kandelia candel*, *Vitex leucoxylon* and *Rhizophora apiculata* plants were selected for phytochemical investigation, *in vitro* antioxidant and anti-inflammatory studies. *Hopea ponga* is an endemic tree belonging to Dipterocarpaceae family found in tropical ever green forest of western India and it is widely distributed along the Western Ghat of Karnataka (Shiddamallayya *et al.*, 2008) [51]. *H. ponga* is categorized as an endangered tree species under the International Union for Conservation of Nature Red List of threatened species. This plant was reported to be used as traditional medicine in the treatment of piles and snake bite (Muralikrishnan & Chandrashekar, 1997) [34]. Bark of *Hopea ponga* is known to have high content of tannin and acts as astringent (Shivaprasad *et al.*, 1999) [52]. Methanolic extract of seed wings of *Hopea ponga* exhibits antioxidant and antibacterial activity (Sukesh *et al.*, 2011) [57]. Literature survey indicates that there are only few pharmacological studies are reported on this plant, revealing the scope for our venture in unmasking its anti-inflammatory property. *Kandelia candel* is the mangrove tree belonging to Rhizophoraceae family which is distributed along the western region of India. *K. candel* whole plant is reported to have antidiabetic activity (Rollet, 1981; Saxena, 1975) [46, 48]. In fact rhizophoraceae species are known to have pharmacological activities. Methanolic extract of *K. candel* is used as anti-hyperglycemic agent in India (Tiwari *et al.*, 2008) and bark, flowers and leaves were reported to have antiviral and antimicrobial properties (Thangam & Kathiresan, 1997; Williams, 1999) [59, 63]. *Vitex leucoxylon* is commonly known as five leaved chaste tree and belongs to the verbenaceae family. It is small to large deciduous tree, growing up to 20 m in height. It is widely distributed along the Western Ghats of India. The leaves of *V. leucoxylon* are reported to have medicinal properties like relieving headache, fever and catarrh (Chanda, 1982) [11]. Reports indicate that aqueous and ethanolic extracts of *V. leucoxylon* leaves possess antipsychotic, antidepressant, analgesic, anti-inflammatory, anti-parkinsonian and antimicrobial activities (Makwana *et al.*, 1994; Sarma *et al.*, 1990) [28, 47]. Even the root and bark of *V. leucoxylon* are reported to use as astringent and febrifuge (Meena *et al.*, 2010) [30]. Many hepatoprotective agents were isolated from leaves and bark of *V. leucoxylon* which includes  $\beta$ -sitosterol, vitexin, isovitexin and aucubin (Rao, 1997). *Rhizophora apiculata* is the tree species of mangrove tree belonging to rhizophoraceae family. In Malaysia, the leaves of *R. apiculata* are assayed as antibreast cancer (Nurhanan *et al.*, 2008) [35]. Studies on HPLC investigation of *R. apiculata* have shown the presence of catechin monomer, a antioxidant flavonoid (Rahim *et al.*, 2008) [41]. This plant is reported to possess anti-inflammatory and anti-tumor properties and is also used to regulate the antioxidant enzymes in biological system (Vinod & Guruvayoorappan, 2014) [62]. Presence of

tannins is reported in the bark of *R. apiculata*, which is known to possess antibacterial and antiviral properties (Sukardjo, 1987; Afidah *et al.*, 2007; Motsei *et al.*, 2003) [56, 1, 33]. Bark of *R. apiculata* is used as a traditional medicine in the treatment of diarrhoea and wounds (School of Thai Traditional Medicine, 1981; Traditional medicine association, 1980). In Malaysia, pyroligneous acid from *R. apiculata* species have been used as sterilizing agent, deodorizer, fertilizer, antimicrobial agent and growth promoting agent (Loo *et al.*, 2006) [26]. Alkaline extract from leaf of *R. apiculata* reported to inhibit the HIV replication and HIV induced cytopathic effects. Some other studies have confirmed the antiviral property of *R. apiculata* extracts, which may be due to presence of anti-polysaccharide in the extracts that acts as an antiviral agent (Kirtikar *et al.*, 1935) [35]. However these four above mentioned plants have not been subjected for investigation for their antioxidant and anti-inflammatory activities. With this background, the present study was undertaken to evaluate the antioxidant and anti-inflammatory properties of *Hopea ponga*, *Kandelia candel*, *Vitex leucoxylon* & *Rhizophora apiculata* plants collected from Western Ghats region of Karnataka, India.

## 2. Materials and methods

### 2.1 Plant Collection

Leaves of *H. ponga*, *K. candel*, *V. leucoxylon* and *R. apiculata* collected from Anashi forest range of Western Ghats, Uttar Kannada District, Karnataka, India during the period of May, 2017. The leaves were identified and authenticated by Dr. Kotresha K, Dept of Botany, Karnatak Science College, Dharwad; Karnataka by referring to the voucher specimen deposited in the Dept of Botany, Karnatak Science College, Dharwad, Karnataka (Voucher specimen No 002, 003, 004 and 005). Fresh plant leaves material was collected and washed under running tap water, shade dried and then homogenized to coarsely powder. The powder was stored in airtight containers at -20 °C for further use for crude solvent extraction.

### 2.2 Crude Extraction

Coarsely powdered dried leaves of *H. ponga*, *K. candel*, *V. leucoxylon* and *R. apiculata* [100g each] were subjected to successive solvent extraction using soxhlet apparatus separately. The extraction of each plant leaves material was done with different solvents in their increasing order of polarity which includes methanol, ethanol and distilled water. Each time the plant material was dried and later extracted with next high polar solvents (following the strategy of extraction in series of increasing the solvent polarity). All extracts were concentrated in Buchi rotoevaporator, followed by removal of traces of solvent by using desiccator.

### 2.3 Phytochemical analysis

The crude extracts (methanol, ethanol and distilled water extracts) of *H. ponga*, *K. candel*, *V. leucoxylon* and *R. apiculata* plants was qualitatively tested for the presence of different phytochemical constituents namely alkaloids, flavonoids, glycosides, phenols, lignin's, saponins, sterols, tannins, anthraquinone and reducing sugar by following the standard procedure reported by Deepti *et al.*, (2012) [13] (Deepti *et al.*, 2012) [13].

### 2.4 Estimation of total phenolic content

The total phenolic content of the *H. ponga*, *K. candel*, *V. leucoxylon* and *R. apiculata* leaf extract was estimated by

using Folin-Ciocalteu method of Singleton *et al.*, (1999) <sup>[53]</sup> (Singleton *et al.*, 1999) <sup>[53]</sup> with slight modification. Gallic acid was used as the reference standard. A volume of 0.5 ml of plant extract was mixed with 2 ml of the Folin-ciocalteu reagent (10 fold) and was neutralized with 4 ml of sodium carbonate solution (8% w/v). The reaction mixture was incubated at room temperature for 30 min for color development. The absorbance of the resulting color was measured at 765 nm using UV-VIS spectrophotometer. The total phenolic contents were estimated from the linear equation of standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent.

### 2.5 Estimation of flavonoids content

The flavonoids content in the plant extracts was estimated according Chang *et al.*, (2002) <sup>[12]</sup> (Chang *et al.*, 2002) <sup>[12]</sup> with quercetin as reference standard. It is an aluminium chloride colorimetric method in which each extract (0.5 ml) separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The reaction mixture was kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm using a UV-VIS Spectrophotometer. The value of optical density was used to calculate the flavonoids content present in the sample and the calibration curve was plotted by using quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

### 2.6 Determination of antioxidant activity by using *in-vitro* methods

#### A) Ferric ion reducing antioxidant power assay (FRAP)

Ferric ions reducing power was measured according to the method of Oyaizu (1986) <sup>[36]</sup> with a slight modification (Oyaizu, 1986) <sup>[36]</sup>. Methanol, ethanol and aqueous extracts of *H. ponga*, *V. leucoxyton*, *R. apiculata* and *K. candel* in different concentrations ranging from 100 µl to 500 µl were mixed with 2.5 ml of 20 mM phosphate buffer and 2.5 ml 1%, w/v potassium ferricyanide, and then the mixture was incubated at 50 °C for 30 min. After incubation, 2.5 ml of 10%, w/v trichloroacetic acid and 0.5 ml 0.1%, w/v ferric chloride were added to the mixture, which was incubated for 10 min. Finally, the absorbance was measured at 700 nm using a UV-VIS Spectrophotometer. Ascorbic acid was used as reference standard. All samples were assayed in triplicates.

#### B) Phosphomolybdenum Assay (PM)

Total antioxidant activity was estimated by Phosphomolybdenum assay using standard procedure of Prieto *et al.*, (1999) <sup>[40]</sup> (Prieto *et al.*, 1999) <sup>[40]</sup>. Methanol, ethanol and aqueous extracts of *H. ponga*, *V. leucoxyton*, *R. apiculata* and *K. candel* in different concentrations ranging from 100 µl to 500 µl were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were incubated at 95 °C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm using a UV-VIS Spectrophotometer. Ascorbic acid was used as reference standard. All samples were assayed in triplicates.

#### C) Hydrogen peroxide scavenging assay:

The antioxidant activity of methanol, ethanol and aqueous extracts of *H. ponga*, *V. leucoxyton*, *R. apiculata* and *K. candel* was evaluated with ascorbic acid as a standard based

on their ability to scavenge the hydrogen peroxide (Gulcin *et al.*, 2002) <sup>[18]</sup>. 0.6 ml of 4mM H<sub>2</sub>O<sub>2</sub> solution in phosphate buffer (pH-7.4) was added to 0.5 ml of known concentration of standard ascorbic acid and to tubes containing different concentrations ranging from 100 µl to 500 µl of plant extracts in phosphate buffer (pH-7.4). Absorbance of the solution was measured at 230 nm after 10 min against the blank solution containing phosphate buffer without hydrogen peroxide. Control was prepared by replacing the sample or standard with phosphate buffer. All samples were assayed in triplicates. The percentage of inhibition was calculated by using formula method.

$$\text{Percentage of inhibition} = A_c - A_t / A_c \times 100$$

#### D) DPPH free radical-scavenging ability assay

Radical scavenging activities of methanol, ethanol and aqueous extracts of *H. ponga*, *V. leucoxyton*, *R. apiculata* and *K. candel* were determined using the DPPH radical as a reagent, according to the methods of Rice-Evans *et al.*, (1997) <sup>[47]</sup> (Rice-Evans *et al.*, 1997) <sup>[47]</sup>. 100 µL of a DPPH radical solution in ethanol (60 µM) was mixed with 100 µL of sample solution in ethanol (different concentrations, w/v). The mixture was incubated for 30 min in the dark at room temperature and then absorbance was measured at 517 nm using a UV-VIS Spectrophotometer. Ascorbic acid was used as a reference standard. The DPPH scavenging activity of each sample was calculated using the following equation:

$$\% \text{ inhibition} = A_c - A_t / A_c \times 100$$

Where, A<sub>c</sub> is the absorbance of the control reaction (100 µL of ethanol with 100 µL of the DPPH solution), and A<sub>t</sub> is the absorbance of the test sample. The experiment was done in triplicate. The IC<sub>50</sub> value was calculated for all the samples. Lower absorbance of the reaction mixture indicated higher free radical activity.

#### E) Evaluation of *in-vitro* anti-inflammatory activity

Anti-inflammatory activity of methanol, ethanol and aqueous extracts of *H. ponga*, *V. leucoxyton*, *R. apiculata* and *K. candel* was evaluated by protein denaturation method as described by Padmanabhan *et al.*, (2012) <sup>[37]</sup> with slight modifications (Padmanabhan *et al.*, 2012) <sup>[37]</sup>. Diclofenac sodium, a powerful non-steroidal anti-inflammatory drug was used as a standard drug. The reaction mixture consisting of 2 mL of different concentrations of methanol and aqueous extract of *H. ponga*, *V. leucoxyton* and *R. apiculata* and methanol, ethanol and aqueous extracts of *K. candel* (500 µg/mL) with standard Diclofenac sodium (500 µg/mL) and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with 2 mL of egg albumin (from fresh hen's egg) and incubated at (27±1) °C for 15 min. Denaturation was induced by keeping the reaction mixture at 70 °C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank. Each experiment was done in triplicate. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = A_t - A_c / A_c \times 100$$

Where, A<sub>t</sub> = absorbance of test sample; A<sub>c</sub> = absorbance of control.

### 2.7 Statistical analysis

All experiments were performed in triplicates (n=3) and the data are presented as the mean ± standard deviation. Differences between the means of the individual groups were analyzed using the analysis of variance procedure of SPSS

software 20 Version (IBM). The significance of differences was defined at the  $p < 0.05$  and  $p < 0.01$  level.

### 3. Results

#### 3.1 Phytochemical Analysis

In the present study, the qualitative phytochemical analysis of methanol, ethanol and aqueous extracts of *H. ponga*, *K. candel*, *V. leucoxylo*n and *R. apiculata* plants was performed by following the standard procedure of various different biochemical tests. In case of *H. ponga* out of three different extracts, phenols, flavonoids and tannins were present in methanol extract, ethanol extract and aqueous extract, glycosides and sterols were detected in all solvent extracts (Table. 1). In case of *K. candel*, alkaloids were detected in methanol and aqueous extracts; whereas methanol, ethanol and aqueous extracts shows the presence of phenols, flavonoids and tannins, among all the three different extracts, presence of lignin was detected in only ethanol extract; glycosides were detected in ethanol and aqueous extracts (Table. 2). In case of *V. leucoxylo*n, among all the three different extracts, phenols and flavonoids in methanol and aqueous extracts; tannins in ethanol and aqueous extracts; glycosides in methanol and ethanol extracts; whereas saponins was absent in all extracts (Table. 3). *R. apiculata* extracts shows variation in all phytochemical contents, among all the three extracts; phenol and flavonoids were detected in methanol and aqueous extracts; tannins were only detected in ethanol extract; glycosides were detected in methanol and ethanol extracts; saponins was absent in all solvent extracts (Table.4).

#### 3.2 Total yield of Crude extract

Phytochemical screening of *H. ponga*, *K. candel*, *V. leucoxylo*n and *R. apiculata* plants shows the variation in phytochemical content and all extracts exhibited as good source of secondary metabolites. The total yield of crude extracts from *H. ponga*, *K. candel*, *V. leucoxylo*n and *R. apiculata* leaves by using the solvents *viz.* methanol, ethanol and aqueous were calculated and shown in Table. 5.

#### 3.3 Total Phenol content

In the present study, total phenolic content of different extracts of *H. ponga*, *K. candel*, *V. leucoxylo*n and *R. apiculata* leaves was determined by the FCR method (Singleton *et al.*, 1999) [53] and expressed as gallic acid equivalents (GAE) per gram of plant extracts. Methanol extract of *K. candel* exhibited highest amount of phenolic content among the extracts i.e  $55.91 \pm 0.0540$  mg/g of gallic acid equivalent. Variation was observed in total phenolic content in each extracts (Table. 6).

#### 3.4 Total Flavonoids content

In the present study, total flavonoids content of different extracts of *H. ponga*, *K. candel*, *V. leucoxylo*n and *R. apiculata* leaves was determined by aluminium chloride colorimetric method (Chang *et al.*, 2002) [12] and expressed as quercetin equivalents (QE) per gram of plant extracts. Aqueous extract of *V. leucoxylo*n exhibited highest amount of flavonoids content ( $53.24 \pm 0.02893$  mg/g) among the all extracts of quercetin equivalent. Variation was observed in total flavonoids content in each extracts (Table. 7).

#### 3.5 FRAP Assay

In the present study, different concentrations of methanol, ethanol and aqueous extracts of *H. ponga*, *V. leucoxylo*n, *R.*

*apiculata* and *K. candel* were subjected to FRAP assay along with ascorbic acid as a standard. Absorbance of the blue colored mixture was measured at 700 nm. The FRAP values were determined as optical density readings. Higher optical density indicates the higher reducing power. In case of *H. ponga* methanol extract of known concentration (500 $\mu$ l) showed good antioxidant activity with absorbance values i.e  $1.2320 \pm 0.00755$ , which was less than the absorbance value of standard ascorbic acid i.e.  $1.4647 \pm 0.00306$ , results are shown in Table. 8. In case of *K. candel*, among methanol, ethanol and aqueous extracts, methanol extract exhibited higher activity among other extracts with absorbance value  $1.2620 \pm 0.00917$  (Table. 9). In FRAP assay of *V. leucoxylo*n methanol, ethanol and aqueous extracts performed along with ascorbic acid as standard. Among these three extracts aqueous extract, aqueous extract showed higher activity than other extracts with absorbance value ( $1.1533 \pm 0.00416$ ) (Table. 10). In case of *R. apiculata*, aqueous extract exhibited significant activity than methanol and ethanol extracts (Table. 11).

#### 3.6 Phosphomolybdenum Assay (PM)

PM assay was used to evaluate total antioxidant capacity of plant extracts along with standard. The antioxidant activity depends on the reducing power of extracts in reduction of phosphate-Mo (VI) at acidic pH. In the present study different concentrations of methanol, ethanol and aqueous extracts of *H. ponga*, *V. leucoxylo*n, *R. apiculata* and *K. candel* along with the ascorbic acid as a standard were used for PM assay. In case of *H. ponga* methanol, ethanol and aqueous extracts showed a very less activity when compared with standard ascorbic acid value ( $1.1313 \pm 0.00702$ ) (Table. 12.). PM assay of methanol, ethanol and aqueous extracts of *K. candel* reveals that among three extracts aqueous extract exhibited highest activity than methanol and ethanol extracts i.e.  $0.9310 \pm 0.00964$  which was near to standard ascorbic value i.e.  $1.1313 \pm 0.00702$  (Table. 13.). In case of *V. leucoxylo*n among methanol, ethanol and aqueous extracts, aqueous extract showed highest activity than methanol extract and standard ascorbic acid i.e.  $1.3863 \pm 0.00252$  (Table. 14.). In case of *R. apiculata*, methanol, ethanol and aqueous extracts were analyzed for antioxidant activity along with ascorbic acid as a standard. The results showed that aqueous extract showed to be having highest activity ( $1.4923 \pm 0.00252$ ) than both methanol extract and standard (Table. 15.).

#### 3.7 Hydrogen peroxide scavenging assay

In hydrogen peroxide radical scavenging assay known concentration (100 $\mu$ g) of methanol, ethanol and aqueous extracts of *H. ponga*, *V. leucoxylo*n, *R. apiculata* and *K. candel* were subjected along with ascorbic acid as a standard. The results revealed that aqueous extract of *V. leucoxylo*n showed highest scavenging activity than standard and among all extracts ( $80.8667 \pm 0.15535$ ). The values of remaining extracts and standard are tabulated in Table. 16.

#### 3.8 DPPH free radical-scavenging ability assay

In the present study, the different concentrations of methanol, ethanol and aqueous extracts of *H. ponga*, *V. leucoxylo*n, *R. apiculata* and *K. candel* were evaluate for their free radical scavenging property by DPPH free radical scavenging assay. The antioxidant capacity of the extracts was compared with ascorbic acid as standard antioxidant. In case of *H. ponga* methanol, ethanol and aqueous extracts shown good scavenging activity but inhibition percentage was less than standard ascorbic acid value ( $83.9100 \pm 0.35000$ ). The results

are shown in Table. 17. The DPPH assay of methanol, ethanol and aqueous extracts of *K. candel* shows that methanol extract exhibited significant scavenging activity than ethanol, aqueous extracts and standard ( $86.0100 \pm 0.30806$ ). The scavenging activity is represented as percentage of inhibition (%) in Table. 18. In case of *V. leucoxyton* among three extracts, methanol and aqueous extracts showed promising antioxidant activity with percentage of inhibition  $81.0733 \pm 0.17786$  and  $83.0200 \pm 0.24021$  respectively (Table.19). In case of *R. sapiculata* among methanol, ethanol and aqueous extract, aqueous extract showed higher antioxidant activity than methanol extract ( $83.5700 \pm 0.12124$ ) (Table. 20).

### 3.9 In-vitro anti-inflammatory assay

Anti-inflammatory study of known concentrations ( $100 \mu\text{g}$ ) of methanol, ethanol and aqueous extracts of *H. ponga*, *V. leucoxyton*, *R. apiculata* and *K. candel* were subjected for anti-inflammatory activity through protein denaturation assay. The *in-vitro* anti-inflammatory activity of the extracts was comparable to the Diclofenac sodium, a reference drug. Significant difference was observed among all extracts in the denaturation of protein. The results revealed that methanol extract of *K. candel* exhibited significant anti-inflammatory activity with percentage of inhibition  $96.9133\%$  than standard drug and all the extracts. Whereas standard drug Diclofenac sodium showed  $94.2467\%$  of inhibition of protein. The results are tabulated in Table. 21.

## 4. Discussion

Plant resources are used as raw material for different purposes with various applications, they not only provide basis needs of life but also they are the valuable source of phytochemicals. Plant derived natural products had great promise for the discovery and development of new drugs. Preliminary screening tests are useful in the detection of different secondary metabolites in the plant crude extracts. Extraction with different solvents with increasing polarity influences the phytoconstituents present in the plants. Hence, in the present study different solvents (methanol, ethanol and aqueous) were used for extraction. In the present study, the phytochemical analysis of *H. ponga*, *K. candel*, *V. leucoxyton* and *R. apiculata* showed the presence of broad spectrum of bioactive compounds such as alkaloids, saponins, tannins, flavonoids and phenols in commonly. These bioactive compounds are reported to have different pharmacological activities like such as anticancer, anti-proliferative, antimicrobial, antioxidant and anti-tuberculosis activities (Sreeram *et al.*, 2005; Supayarg *et al.*, 2004) [54, 58]. Several studies show that alkaloids, glycosides are known to possess antimicrobial properties (Balandrin & Klocke, 1988) [6]. In the present study, methanol extract of *H. ponga*; methanol, ethanol and aqueous extracts of *K. candel*; showed the presence of alkaloids. Saponins are considered as key ingredient to produce inhibitory effect on inflammation, they also used as dietary supplements and nutraceuticals. Different physical and biological properties of saponins made them useful drugs such as antimicrobial, anti-inflammatory and hemolytic agent (George *et al.*, 2002; Xu *et al.*, 1996) [16, 64]. In the present investigation, methanol extract of *H. ponga* and *V. leucoxyton* only showed the presence of saponins, where as it was absent in remaining plant extracts. Many tannin drugs are being used as astringent. They are also used as healing agent, anti-inflammatory agent, and anti-toed and also against burns and piles. Tannins are considered to be possessing medicinal properties like antiviral, antibacterial,

anti-parasitic, anticancer, antiulcer and antioxidant agents. It also investigated that this was able to inhibit HIV replication (Lv *et al.*, 2004; Akiyama *et al.*, 2001; Kolodziej & Kiderlen, 2005) [26, 2, 25]. In the present study, methanol and ethanol extracts of *H. ponga* and *V. leucoxyton*; methanol, ethanol and aqueous extracts of *K. candel* showed the presence of tannins, whereas in case of *R. apiculata* it was found to be absent in all extracts. Many literatures and extensive investigation indicates that the plant phenolics are the major primary compounds which are acting as free radical scavengers. Even the flavonoids are considered to be diverse and broad group of natural components which possess broad spectrum of biological activities including antioxidant activity (Blois, 1958) [9]. In the present study, methanol and ethanol extracts of *H. ponga*; methanol, ethanol and aqueous extracts of *K. candel*; methanol and aqueous extracts of *V. leucoxyton* and *R. apiculata* showed the presence of phenols. On consideration with flavonoids methanol extract of *H. ponga*; methanol, ethanol and aqueous extracts of *K. candel*; methanol and aqueous extracts of *V. leucoxyton* and *R. apiculata* showed the presence of flavonoids. In the present study, the total phenolic content and flavonoids content was also calculated. Methanol extract of *K. candel* exhibited highest amount of phenolic content among all the extracts ( $55.91 \pm 0.0540$  mg/g) of gallic acid equivalent and aqueous extract of *V. leucoxyton* exhibited highest amount of flavonoids content among all the extracts ( $53.24 \pm 0.02893$  mg/g) of quercetin equivalent. Phenolic compounds and flavonoids can play the role of antioxidant through different mechanisms including terminating free radicals, reducing the oxygen concentration, transforming primary products of oxidation into non oxidant molecules and acts as metal chelators (Shahidi & Nacz, 2004) [50].

Free radicals are the chemical species which contains one or more unpaired electrons. These are highly unstable and attain stability cause damage to other molecules by extracting electrons from them. These free radicals enhances the abnormal uncontrolled oxidation reaction in the body which leads to the failure of antioxidant defense mechanism and causes damage to the cell structures which increases the risk factors for many diseases such as Alzheimer's disease, Parkinson disease, cardiovascular disorders, liver disease, inflammation and cancer (Rajkapoor *et al.*, 2010) [42]. In the antioxidant defense system, enzymatic antioxidant such as superoxide dismutase (SO), catalase (CAT), glutathione peroxidase (CPx) and non-enzymatic plant derived antioxidants such as caretenoids, ascorbic acid, phenol, flavonoids etc., are having antioxidant capacity acts as scavengers in the living system. Risk of the chronic disease and its progression can be achieved by increasing natural antioxidant defense or supplementing with the proven antioxidants (Rajkumar *et al.*, 2010) [43]. Antioxidant mechanisms in biological tissues are extremely complex and by the only one method it is difficult to decide the antioxidant capacity of crude extracts (Carocho & Ferreira, 2013) [10]. A large number of *in-vitro* methods are available to evaluate the antioxidant activity of pure compound or extracts. Hence in the present study, four *in-vitro* assays *viz.*, FRAP, PM,  $\text{H}_2\text{O}_2$  and DPPH assay are employed.

FRAP assay includes use of ferricyanide and ferric ions as chromogenic oxidants. It gives an immediate result of a large range of individual antioxidants in dose dependent manner and intensity of color directly proportional to the reducing power of antioxidants. The color of the reaction mixture which measured at 700nm and higher the absorbance

indicates higher antioxidant activity. In the present study methanol extract of *K. candel* and aqueous extract of *R. apiculata* exhibited higher activity over all extracts with absorbance  $1.2620 \pm 0.00917$  and  $1.2903 \pm 0.00709$  respectively.

PM assay is a quantitative method used to evaluate redox reaction by antioxidant, oxidants with the involvement of ligand molybdenum. It involves the longer incubation period at higher temperature which influences the auto-oxidation reaction in the mixture. It directly estimates the reducing potent of the extracts. The reaction mixture forms green Phosphomolybdenum colored complex at acidic pH which is measured at 695nm. The reduction activity of extracts and standard drug was increased with increasing in concentrations. In the present study, aqueous extract of *V. leucoxyton* and *R. apiculata* possessed higher activity ((1.3863 and 1.4923 respectively) which was higher than the standard and remaining extracts.

$H_2O_2$  assay is one of the common methods used to investigate the antioxidant capacity of the extracts. Hydrogen peroxide is very inactive but in some cases it causes adverse effects to cell by releasing hydroxyl radicals in the cells and become toxic to the cells.  $H_2O_2$  can be removed by antioxidants such as phenols, polyphenols and flavonoids (Esmaili & Sonboli, 2010) [15] and protects mammalian cells from damage induced by hydrogen peroxide. In this assay, scavenging activity of extracts was detected and compared with ascorbic acid which is taken as reference standard. In  $H_2O_2$  assay strong oxidizing agent which inactivate certain enzymes directly and also react

with metal ions like  $Fe^{+2}$ ,  $Cu^{+2}$  and leads to the toxic effects (Bhatia *et al.*, 2011) [8]. In the present study, aqueous extract of *V. leucoxyton* exhibited highest scavenging activity with the 80.8667% percentage of inhibition on comparing with standard and remaining plants extracts.

DPPH assay is most widely accepted method for evaluating antioxidant activity of many plant based drugs and crude extracts (Philips *et al.*, 2010) [38]. It is based on the reduction of methanolic solution of colored free radical DPPH by free radical scavengers. Concentration of free radical scavengers is proportional to scavenging DPPH with the absorbance at 517 nm. The reducing potential is measured by decreasing in absorbance by the action extracts. In the present study, aqueous extract of *V. leucoxyton* and *R. apiculata* showed higher antioxidant activity with 83.0200 and 83.5700 percentage of inhibition which was comparable to inhibition percentage of standard (83.9100).

Inflammation is a common reaction of living tissues towards infection and injuries (Mohamed *et al.*, 2011) [32]. Steroidal anti-inflammatory agents are available but they induce damage to the lymphocytes and causes severe side effects, so the anti-inflammatory agents from natural sources like plants getting more importance and they are more promising agent with less side effect. In the present study, *in-vitro* protein denaturation method was used in which methanol extract of *K. candel* showed to be exhibit highest anti-inflammatory activity than the standard drug and all extracts with 96.9133 percentage inhibition of the protein denaturation.

**Table 1:** Phytochemical constituents present in the *H. pongaleaves*

Tests	Methanol extract	Ethanol extract	Aqueous extract
A) Alkaloids			
Iodine	-	-	-
Wagner's	+	-	-
Dragendorff's	+	-	-
B) Flavonoids			
Pew's Test	++	+	-
Shinoda Test	++	++	-
NaoH Test	++	++	-
C) Glycosides			
K-K Test	+	++	++
Glycoside Test	+	++	++
Conc. $H_2SO_4$ Test	+	++	++
Molish Test	+	++	+
D) Phenols			
Ellagic acid Test	+	+	-
Phenols Test	+	+	-
E) Lignin			
Lignin Test	-	-	-
Lobat Test	-	-	-
F) Saponins			
Foam Test	+	-	-
Haemolysis Test	++	-	-
G) Sterols			
L-B Test	+	+	+
Salkowsk Test	+	+	+
H) Tannins			
Gelatin Test	+	+	-
Lead acetate Test	++	++	-
I) Anthraquinone			
Bomtrager's Test	-	-	-
J) Phlobatanin	-	-	-
K) Oils and Fats			
Filter paper Test	++	++	++
Saponification Test	+	-	++

-: Absent; +: Moderate; ++: High presence.

**Table 2:** Phytochemical constituents present in the *K. candelleaves*

Tests	Methanol extract	Ethanol extract	Aqueous extract
<b>A) Alkaloids</b>			
Iodine	-	-	+
Wagner's	+	-	+
Dragendorff's	+	-	+
<b>B) Flavonoids</b>			
Pew's Test	++	-	-
Shinoda Test	++	-	-
NaOH Test	++	-	-
<b>C) Glycosides</b>			
K-K Test	-	+	++
Glycoside Test	-	+	++
Conc. H <sup>2</sup> SO <sub>4</sub> Test	-	+	++
Molish Test	-	+	+
<b>D) Phenols</b>			
Ellagic acid Test	++	++	+
Phenols Test	++	++	+
<b>E) Lignin</b>			
Lignin Test	-	+	-
Lobat Test	-	+	-
<b>F) Saponins</b>			
Foam Test	-	-	-
Haemolysis Test	-	-	+
<b>G) Sterols</b>			
L-B Test	+	+	+
Salkowsk Test	+	+	+
<b>H) Tannins</b>			
Gelatin Test	+	+	+
Lead acetate Test	++	++	+
<b>I) Anthraquinone</b>			
Bomtrager's Test	-	-	-
<b>J) Phlobatanin</b>			
	-	-	-
<b>K) Oils and Fats</b>			
Filter paper Test	+	-	+
Saponification Test	+	-	++

-: Absent; +: Moderate; ++: High presence.

**Table 3:** Phytochemical constituents present in the *V. leucoxyton* leaves

Tests	Methanol extract	Ethanol extract	Aqueous extract
<b>A) Alkaloids</b>			
Iodine	-	-	-
Wagner's	+	+	-
Dragendorff's	+	+	-
<b>B) Flavonoids</b>			
Pew's Test	++	+	+
Shinoda Test	++	-	++
NaOH Test	++	+	+
<b>C) Glycosides</b>			
K-K Test	+	+	-
Glycoside Test	+	+	-
Conc. H <sup>2</sup> SO <sub>4</sub> Test	+	+	-
Molish Test	+	+	-
<b>D) Phenols</b>			
Ellagic acid Test	++	++	-
Phenols Test	-	-	-
<b>E) Lignin</b>			
Lignin Test	-	-	-
Lobat Test	+	-	-
<b>F) Saponins</b>			
Foam Test	++	-	-
Haemolysis Test	++	-	-
<b>G) Sterols</b>			
L-B Test	-	-	-
Salkowsk Test	-	+	-
<b>H) Tannins</b>			
Gelatin Test	+	+	-

Lead acetate Test	+	++	-
I] Anthraquinone			
Bomtrager's Test	-	-	-
J] Phlobatanin	-	-	-
K] Oils and Fats			
Filter paper Test	-	+	+
Saponification Test	+	-	++

-: Absent; +: Moderate; ++: High presence.

**Table 4:** Phytochemical constituents present in the *R. apiculata* leaves

TESTS	Methanol extract	Ethanol extract	Aqueous extract
A] Alkaloids			
Iodine	-	-	-
Wagner's	+	-	-
Dragendorff's	-	-	-
B] Flavonoids			
Pew's Test	-	-	++
Shinoda Test	-	-	+
NaoH Test	-	-	++
C] Glycosides			
K-K Test	+	+	-
Glycoside Test	+	+	-
Conc. H <sup>2</sup> SO <sub>4</sub> Test	+	+	+
Molish Test	+	+	-
D] Phenols			
Ellagic acid Test	++	++	++
Phenols Test	++	++	++
E] Lignin			
Lignin Test	-	-	-
Lobat Test	-	-	-
F] Saponins			
Foam Test	-	-	-
Haemolysis Test	-	-	+
G] Sterols			
L-B Test	+	+	++
Salkowsk Test	-	-	+
H] Tannins			
Gelatin Test	-	+	-
Lead acetate Test	-	++	-
I] Anthraquinone			
Bomtrager's Test	-	-	-
J] Phlobatanin	-	-	-
K] Oils and Fats			
Filter paper Test	-	++	+
Saponification Test	+	-	-

-: Absent; +: Moderate; ++: High presence.

**Table 5:** Total yield of different solvents extract of experimental plants

Sl. No.	Sample	Total yield
1.	<i>H. ponga</i> methanol extract	4%
2.	<i>H. ponga</i> ethanol extract	2.9%
3.	<i>H. ponga</i> aqueous extract	5%
4.	<i>K. candel</i> methanol extract	5.7%
5.	<i>K. candel</i> ethanol extract	4%
6.	<i>K. candel</i> aqueous extract	3.5%
7.	<i>V. leucoxydon</i> methanol extract	5.7%
8.	<i>V. leucoxydon</i> ethanol extract	6.5%
9.	<i>V. leucoxydon</i> aqueous extract	7.5%
10.	<i>R. apiculata</i> methanol extract	5.4%
11.	<i>R. apiculata</i> ethanol extract	5%
12.	<i>R. apiculata</i> aqueous extract	8%

**Table 6:** Total Phenol content in different solvent extracts of experimental plants

S. No.	Sample	Total phenolic content (mg)
1	<i>X. americana</i> methanol extract	88.30 ± 0.14
2	<i>X. americana</i> aqueous extract	91.40 ± 0.14
3	<i>H. ponga</i> methanol extract	29.5495±0.15368
4	<i>H. ponga</i> ethanol extract	34.0630±0.06800
5	<i>K. candel</i> methanol extract	55.9189±0.05405
6	<i>K. candel</i> ethanol extract	14.4144±0.10927
7	<i>K. candel</i> aqueous extract	31.5495±0.10922
8	<i>V. leucoxydon</i> methanol extract	40.2252± 0.06799
9	<i>V. leucoxydon</i> aqueous extract	44.1801± 0.08691
10	<i>R. apiculata</i> chloroform extract	20.1171±0.09492
11	<i>R. apiculata</i> methanol extract	40.1171±0.10922
12	<i>R. apiculata</i> ethanol extract	22.1171±0.08254
13	<i>R. apiculata</i> aqueous extract	44.9549±0.05630

**Table 7:** Total flavonoids content in different solvent extracts of experimental plants

Sl. No.	Sample	Total flavonoids content (mg)
1	<i>X. americana</i> methanol extract	38.20 ± 0.12
2	<i>X. americana</i> aqueous extract	61.40 ± 0.173



3	<i>H. ponga</i> methanol extract	39.6350±0.12165
4	<i>H. ponga</i> ethanol extract	23.8523±0.17088
5	<i>K. candel</i> methanol extract	48.8889±0.05064
6	<i>V. leucoxyton</i> methanol extract	52.1573± 0.06121
7	<i>V. leucoxyton</i> aqueous extract	53.2450± 0.02893
8	<i>R. apiculata</i> aqueous extract	51.7923±0.05064

**Table 8:** FRAP Assay of *Hopea ponga* extracts

Sl.no	Concentration	Std Ascorbic acid	Methanol extract	Ethanol extract	Aqueous extract
1	100µl	0.4650±0.00300*	0.5527±0.00503*	0.4367±0.00416*	0.2220±0.01000*
2	200 µl	0.7530±0.00458*	0.7177±0.00351*	0.6133±0.00802*	0.3303±0.01550*
3	300 µl	0.9943±0.00416*	0.8433±0.00611*	0.8167±0.00611*	0.4907±0.00611*
4	400 µl	1.1353±0.00416**	0.9467±0.00416*	0.8743±0.00950*	0.5793±0.00416*
5	500 µl	1.4647±0.00306**	1.2320±0.00755**	1.0247±0.00503*	0.6427±0.00503*

Correlation is significant at the 0.01 level (2-tailed)\*\*

Correlation is significant at the 0.05 level (2-tailed)\*

**Table 9:** FRAP Assay of *K. candel* extracts

Sl. no	Concentration	Std Ascorbic acid	Methanol extract	Ethanol extract	Aqueous extract
1	100µl	0.4650±0.00300**	0.3490±0.00265**	0.2687±0.00503**	0.2917±0.00351*
2	200 µl	0.7530±0.00458**	0.4310±0.00361**	0.3947±0.00306**	0.4433±0.00416*
3	300 µl	0.9943±0.00416**	0.6737±0.01457**	0.5120±0.00200**	0.5797±0.00153*
4	400 µl	1.1353±0.00416**	0.8617±0.00751**	0.6433±0.00416**	0.7710±0.00964*
5	500 µl	1.4647±0.00306**	1.2620±0.00917**	0.7160±0.00529**	0.8867±0.00379*

Correlation is significant at the 0.01 level (2-tailed)\*\*

Correlation is significant at the 0.05 level (2-tailed)\*

**Table 10:** FRAP Assay of *Vitex leucoxyton* extracts

Sl.no	Concentration	Std Ascorbic acid	Methanol extract	Ethanol extract	Aqueous extract
1	100µl	0.4650±0.00300*	0.2653±0.00321*	0.3327±0.00702*	0.3890±0.00300*
2	200 µl	0.7530±0.00458*	0.5543±0.00321*	0.4513±0.00833*	0.5783±0.00252*
3	300 µl	0.9943±0.00416*	0.7463±0.00379*	0.5287±0.00902*	0.6870±0.00458*
4	400 µl	1.1353±0.00416*	0.8767±0.00451*	0.6680±0.00872*	1.0417±0.00252*
5	500 µl	1.4647±0.00306**	1.0113±0.00404*	0.7820±0.00400*	1.1533±0.00416*

Correlation is significant at the 0.01 level (2-tailed)\*\*

Correlation is significant at the 0.05 level (2-tailed)\*

**Table 11:** FRAP Assay of *Rhizophora apiculata* extracts

Sl.no	Concentration	Std Ascorbic acid	Methanol extract	Ethanol extract	Aqueous extract
1	100µl	0.4650±0.00300*	0.5747±0.00306*	0.3653±0.00252*	0.4840±0.00300*
2	200 µl	0.7530±0.00458*	0.7083±0.00351*	0.4417±0.00351*	0.5987±0.00503*
3	300 µl	0.9943±0.00416*	0.8377±0.00351*	0.5823±0.00379*	0.8237±0.00451*
4	400 µl	1.1353±0.00416**	0.8620±0.00200*	0.6940±0.00529*	0.9187±0.00252*
5	500 µl	1.4647±0.00306**	1.1123±0.00153*	0.7847±0.00503*	1.2903±0.00709**

Correlation is significant at the 0.01 level (2-tailed)\*\*

Correlation is significant at the 0.05 level (2-tailed)\*

**Table 12:** PM Assay of *Hopea ponga* extracts

Sl.no	Concentration	Std Ascorbic acid	Methanol extract	Ethanol extract	Aqueous extract
1	100µl	0.2780±0.00400*	0.1397±0.00208*	0.1893±0.00611	0.1067±0.00503*
2	200 µl	0.5020±0.00400*	0.3113±0.00208*	0.2113±0.00764	0.1993±0.00416*
3	300 µl	0.6810±0.00458*	0.4543±0.00252*	0.2310±0.00361	0.2407±0.00643*
4	400 µl	0.9017±0.00351*	0.5873±0.00351*	0.3300±0.00361	0.3907±0.00416*
5	500 µl	1.1313±0.00702**	0.6420±0.00600*	0.4520±0.00400*	0.4420±0.00529*

Correlation is significant at the 0.01 level (2-tailed)\*\*

Correlation is significant at the 0.05 level (2-tailed)\*

**Table 13:** PM Assay of *K. candell* extracts

Sl.no	Concentration	Std Ascorbic acid	Methanol extract	Ethanol extract	Aqueous extract
1	100µl	0.2780±0.00400*	0.1407±0.00306*	0.1130±0.00458*	0.3403±0.00802*
2	200 µl	0.5020±0.00400*	0.2530±0.00265*	0.3087±0.00416*	0.4860±0.00458*
3	300 µl	0.6810±0.00458*	0.3443±0.00404*	0.4270±0.00265*	0.5740±0.00346*
4	400 µl	0.9017±0.00351*	0.4140±0.00346*	0.5597±0.00252*	0.7567±0.00451*
5	500 µl	1.1313±0.00702**	0.6853±0.00153*	0.5753±0.00651*	0.9310±0.00964*

Correlation is significant at the 0.01 level (2-tailed)\*\*

Correlation is significant at the 0.05 level (2-tailed)\*

**Table 14:** PM Assay of *Vitex leucoxylo* extracts

Sl.no	Concentration	Std Ascorbic acid	Methanol extract	Ethanol extract	Aqueous extract
1	100µl	0.2780±0.00400*	0.1960±0.00200	0.1333±0.00611	0.2983±0.00252*
2	200 µl	0.5020±0.00400*	0.3073±0.00306	0.2420±0.00800	0.4723±0.00153*
3	300 µl	0.6810±0.00458*	0.3647±0.00306*	0.3680±0.00400	0.8940±0.00200*
4	400 µl	0.9017±0.00351*	0.4660±0.00200*	0.4700±0.00346*	1.2933±0.00231**
5	500 µl	1.1313±0.00702**	0.5947±0.00306*	0.5380±0.00529*	1.3863±0.00252**

Correlation is significant at the 0.01 level (2-tailed)\*\*

Correlation is significant at the 0.05 level (2-tailed)\*

**Table 15:** PM Assay of *Rhizophora apiculata* extracts

Sl.no	Concentration	Std Ascorbic acid	Methanol extract	Ethanol extract	Aqueous extract
1	100µl	0.2780±0.00400	0.1830±0.00557	0.1073±0.00503	0.3197±0.00512
2	200 µl	0.5020±0.00400*	0.2343±0.00252	0.1953±0.00306	0.6250±0.00265*
3	300 µl	0.6810±0.00458*	0.4463±0.00208*	0.2623±0.00379	0.8433±0.00321*
4	400 µl	0.9017±0.00351*	0.5770±0.00265*	0.3807±0.00306	1.1257±0.00153**
5	500 µl	1.1313±0.00702**	0.6537±0.00153*	0.4853±0.00416*	1.4923±0.00252**

Correlation is significant at the 0.01 level (2-tailed)\*\*

Correlation is significant at the 0.05 level (2-tailed)\*

**Table 16:** H<sub>2</sub>O<sub>2</sub> Assay of different solvent extracts of experimental plants

Sl. No.	Concentration	Treatment	% Inhibition
1	100 µg	Standard	74.4633±0.13051
2	100 µg	<i>H. ponga</i> methanol extract	64.8267±0.07506
3	100 µg	<i>H. ponga</i> ethanol extract	58.2333±0.11504
4	100 µg	<i>H. ponga</i> aqueous extract	54.4444±0.21297
4	100 µg	<i>K. candel</i> methanol extract	70.8167±0.07506
5	100 µg	<i>K. candel</i> ethanol extract	35.3167±0.10017
6	100 µg	<i>K. candel</i> aqueous extract	39.6467±0.11240
7	100 µg	<i>V. leucoxylo</i> methanol extract	65.1000±0.09539
8	100 µg	<i>V. leucoxylo</i> ethanol extract	54.3321±0.15592
9	100 µg	<i>V. leucoxylo</i> aqueous extract	80.8667±0.15535
10	100 µg	<i>R. apiculata</i> methanol extract	69.4700±0.13454
11	100 µg	<i>R. apiculata</i> ethanol extract	58.3770±0.18849
12	100 µg	<i>R. apiculata</i> aqueous extract	73.2267±0.11676

**Table 17:** Anti-inflammatory Assay of different solvent extracts of experimental plants

Sl. No.	Concentration	Treatment	% Inhibition
1	100 µg	Standard	94.2467±1.90148
2	100 µg	<i>H. ponga</i> methanol extract	69.5133±1.41394
3	100 µg	<i>H. ponga</i> ethanol extract	20.1000±1.63000
4	100 µg	<i>H. ponga</i> aqueous extract	31.1594±1.66032
5	100 µg	<i>K. candel</i> methanol extract	96.9133±1.66064
6	100 µg	<i>K. candel</i> ethanol extract	12.3133±2.79185
7	100 µg	<i>K. candel</i> aqueous extract	54.5233±3.53856
8	100 µg	<i>V. leucoxylo</i> methanol extract	34.7767±2.17500
9	100 µg	<i>V. leucoxylo</i> ethanol extract	16.4855±1.13139
10	100 µg	<i>V. leucoxylo</i> aqueous extract	54.8867±1.95809
11	100 µg	<i>R. apiculata</i> methanol extract	27.3500±1.90638
12	100 µg	<i>R. apiculata</i> ethanol extract	21.9202±1.90866
13	100 µg	<i>R. apiculata</i> aqueous extract	77.8933±1.66064

**Table 18:** Percentage inhibition of DPPH free radical of *Hopea ponga* extracts

Sl.no	Concentration	Std Ascorbic acid	Methanol extract	Ethanol extract	Aqueous extract
1	10µg	67.7867±0.17898*	51.6300±0.35000	57.0633±0.52596	42.7350±0.81862
2	20 µg	73.6533±0.23502*	57.4500±0.35000*	58.9300±0.41146*	55.6332±0.48524*
3	30 µg	78.2000±0.30806**	69.8900±0.29206*	69.4233±0.44287*	62.9370±0.23310*
4	40 µg	80.8000±0.35679**	73.1100±0.35679**	75.3667±0.24420**	66.9386±0.52558**
5	50 µg	83.9100±0.35000**	77.2700±0.35000**	78.9000±0.42297**	74.8251±0.46620**

Correlation is significant at the 0.01 level (2-tailed)\*\*

Correlation is significant at the 0.05 level (2-tailed)\*

**Table 19:** Percentage inhibition of DPPH free radical of *K. candel* extracts

Sl.no	Concentration	Std Ascorbic acid	Methanol extract	Ethanol extract	Aqueous extract
1	10µg	67.7867±0.17898*	73.8867±0.46501**	58.2300±0.29206*	68.7200±0.41146*
2	20 µg	73.6533±0.23502**	77.3067±0.35572**	65.6500±0.35679*	72.8533±0.32347**

3	30 µg	78.2000±0.30806**	80.7100±0.19975**	68.2700±0.15100*	74.8967±0.48840**
4	40 µg	80.8000±0.35679**	84.0200±0.73980**	71.6367±0.24420*	77.4233±0.17898**
5	50 µg	83.9100±0.35000**	86.0100±0.30806**	73.6267±0.56889**	78.7833±0.23502**

Correlation is significant at the 0.01 level (2-tailed)\*\*

Correlation is significant at the 0.05 level (2-tailed)\*

**Table 20:** Percentage inhibition of DPPH free radical of *V. leucoxydon* extracts

Sl.no	Concentration	Std Ascorbic acid	Methanol extract	Ethanol extract	Aqueous extract
1	10µg	67.7867±0.17898*	57.8800±0.35679	46.0761±0.58662	68.2167±0.35572*
2	20 µg	73.6533±0.23502*	67.2033±0.35572*	59.4794±0.59808	70.1167±0.37434**
3	30 µg	78.2000±0.30806**	72.5700±0.29206**	60.5112±0.48524	75.9067±0.27135**
4	40 µg	80.8000±0.35679**	78.3567±0.40624**	72.4941±0.46620*	80.3333±0.24420**
5	50 µg	83.9100±0.35000**	81.0733±0.17786**	78.0108±0.48524**	83.0200±0.24021**

Correlation is significant at the 0.01 level (2-tailed)\*\*

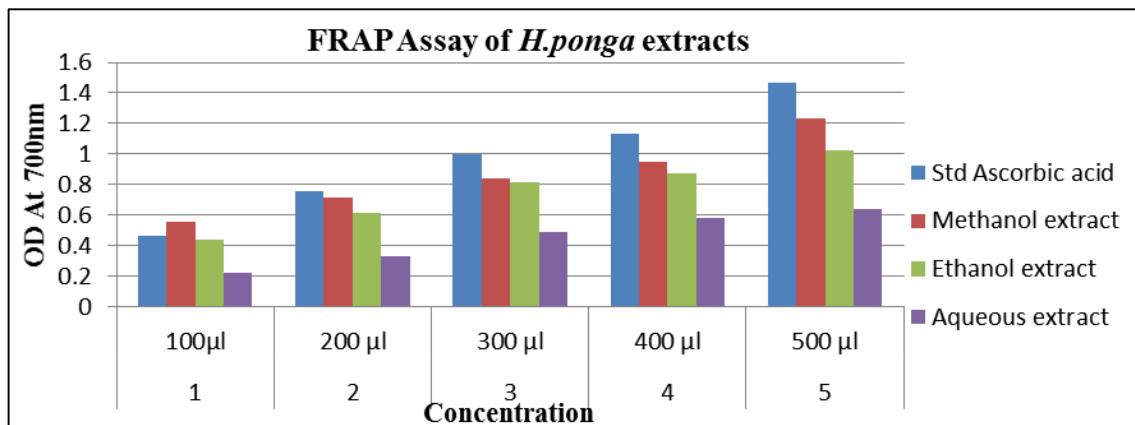
Correlation is significant at the 0.05 level (2-tailed)\*

**Table 21:** Percentage inhibition of DPPH free radical of *Rhizophora apiculata* extracts

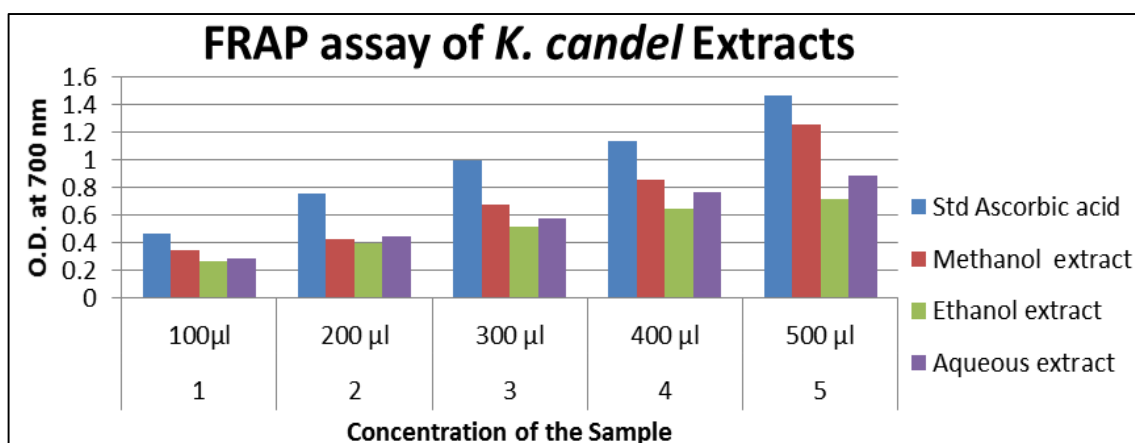
Sl.no	Concentration	Std Ascorbic acid	Methanol extract	Ethanol extract	Aqueous extract
1	10µg	67.7867±0.17898*	61.4167±0.53463*	39.8601±0.46620	66.8533±0.40624*
2	20 µg	73.6533±0.23502*	65.7300±0.47000*	48.7179±0.23308	71.5200±0.41146*
3	30 µg	78.2000±0.30806**	70.6633±0.37434*	58.2750±0.69930	78.0067±0.48439**
4	40 µg	80.8000±0.35679**	74.9400±0.35000**	66.4335±0.46620*	81.2700±0.35679**
5	50 µg	83.9100±0.35000**	78.1200±0.43936**	74.6697±0.37465**	83.5700±0.12124**

Correlation is significant at the 0.01 level (2-tailed)\*\*

Correlation is significant at the 0.05 level (2-tailed)\*



**Fig 1:** FRAP assay of *Hopea ponga* extracts



**Fig 2:** FRAP assay of *K. candel* extracts

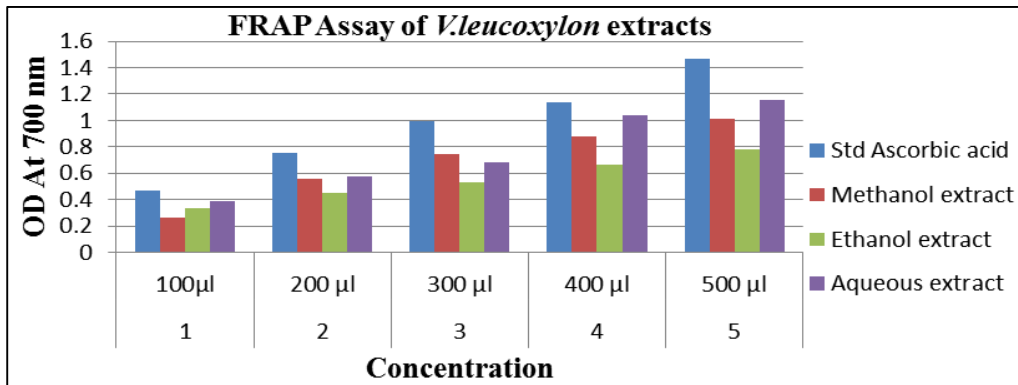


Fig 3: FRAP assay of *Vitex leucoxylon* extracts

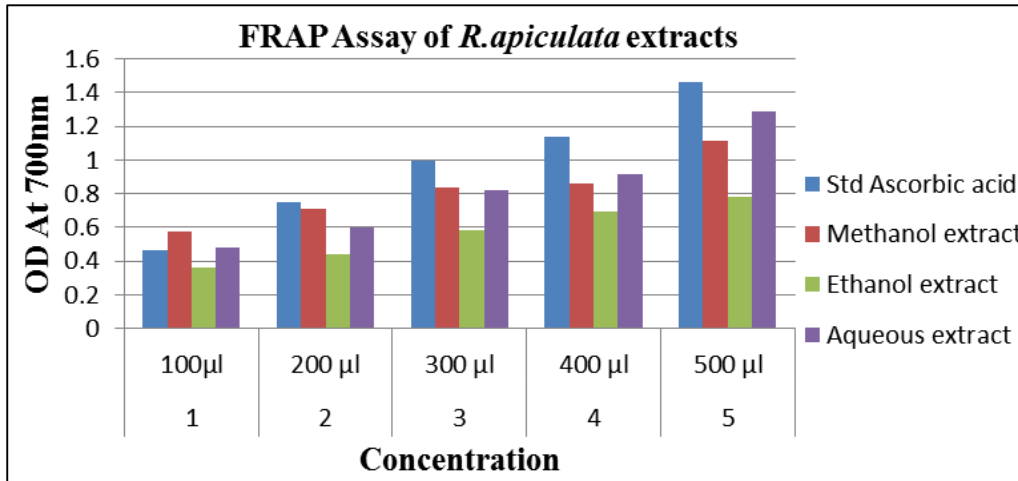


Fig 4: FRAP assay of *Rhizophora apiculata* extracts

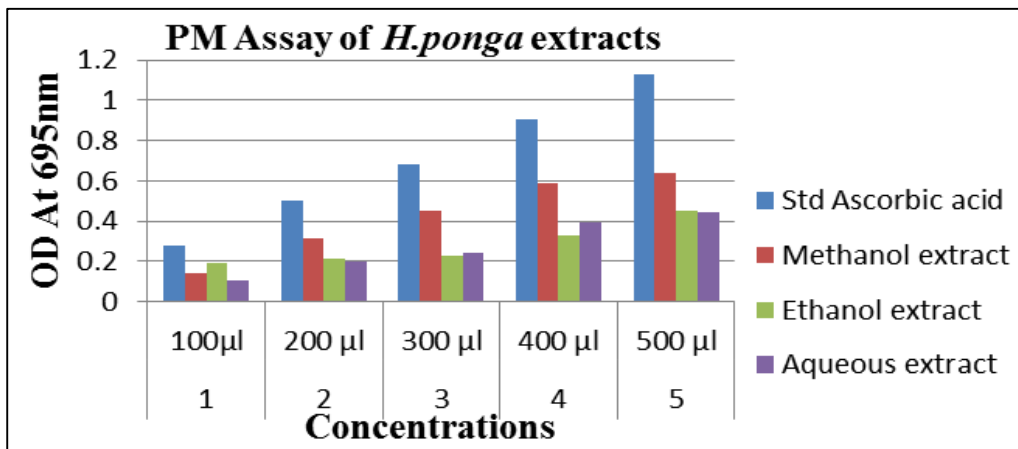


Fig 5: PM Assay of *Hopea ponga* extracts

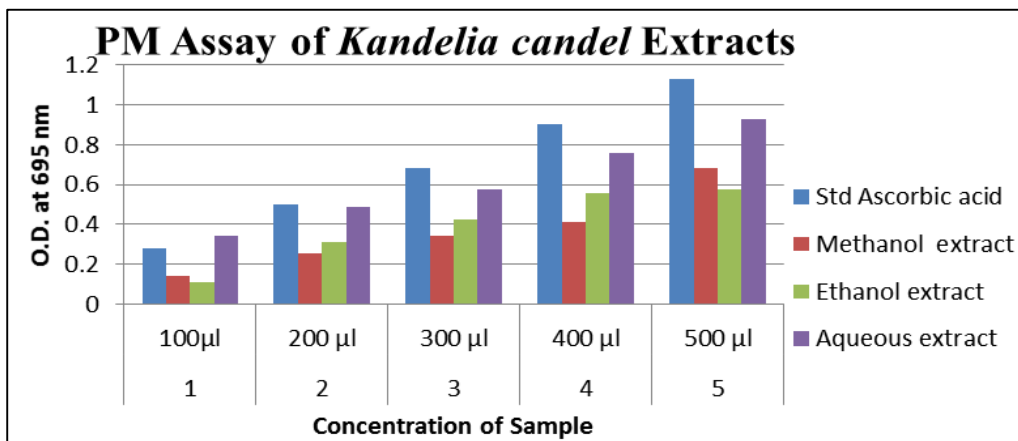


Fig 6: PM assay of *K. candel* extracts

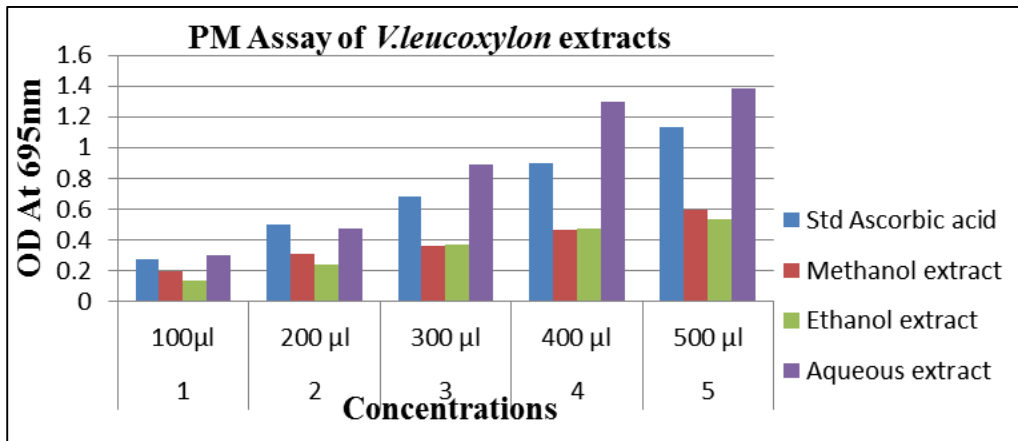


Fig 7: PM Assay of *Vitex leucoxyylon* extracts

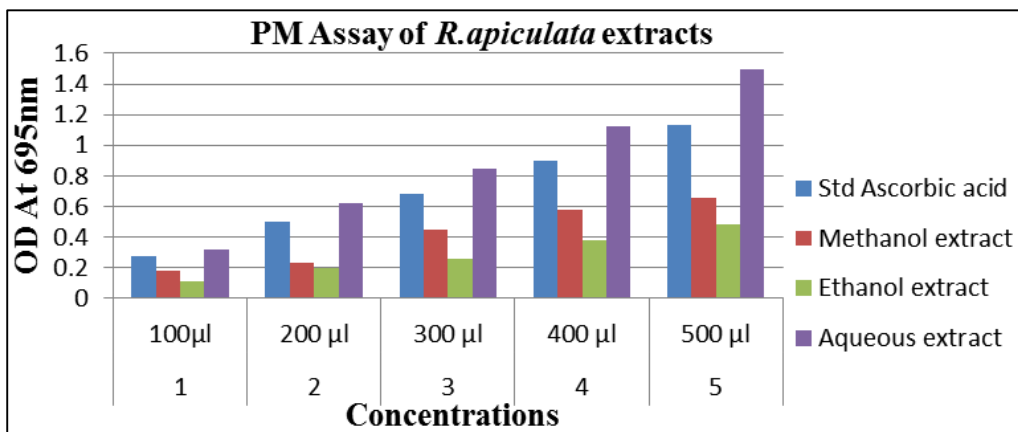


Fig 8: PM Assay of *Rhizophora apiculata* extracts

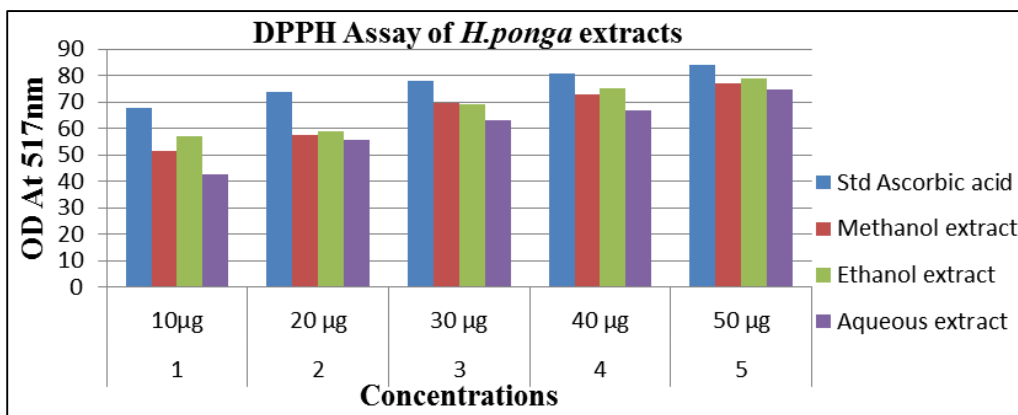


Fig 9: Percentage inhibition of DPPH free radical of *Hopea ponga* extracts

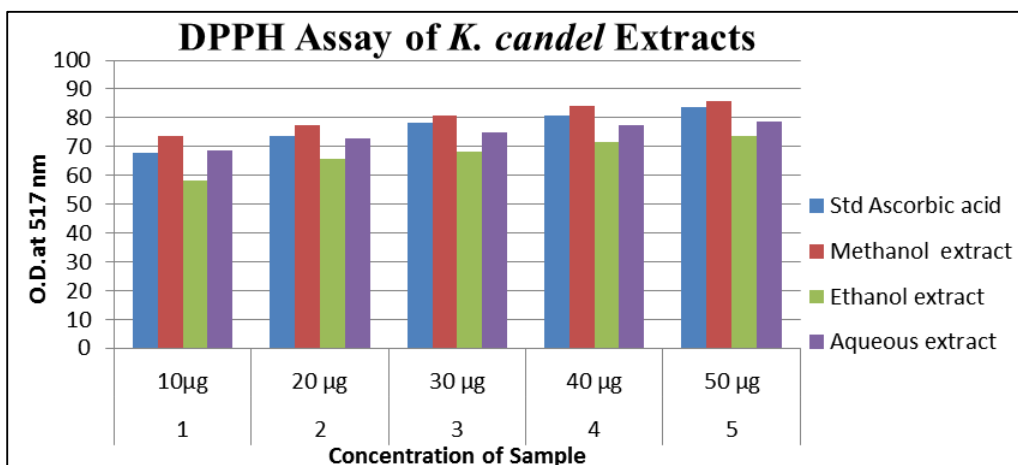


Fig 10: Percentage inhibition of DPPH free radical of *K. candel* extracts

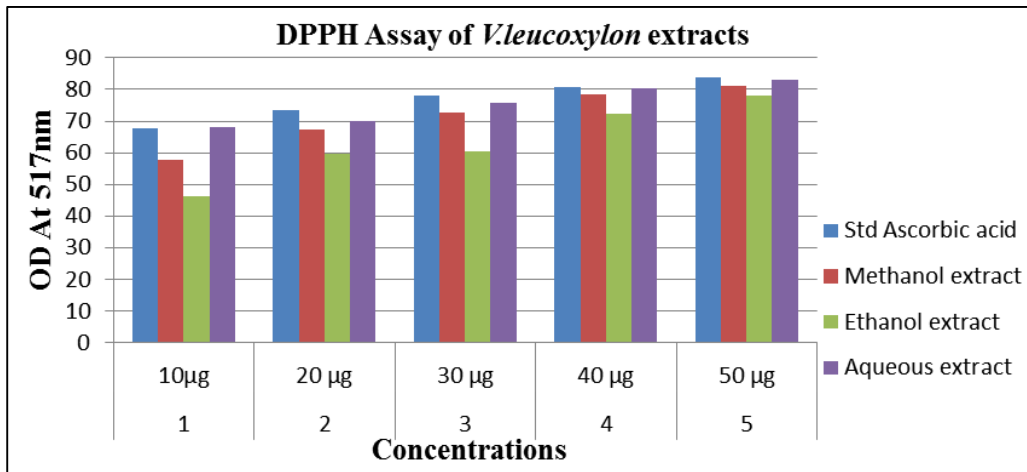


Fig 11: Percentage inhibition of DPPH free radical of *V. leucoxydon* extracts

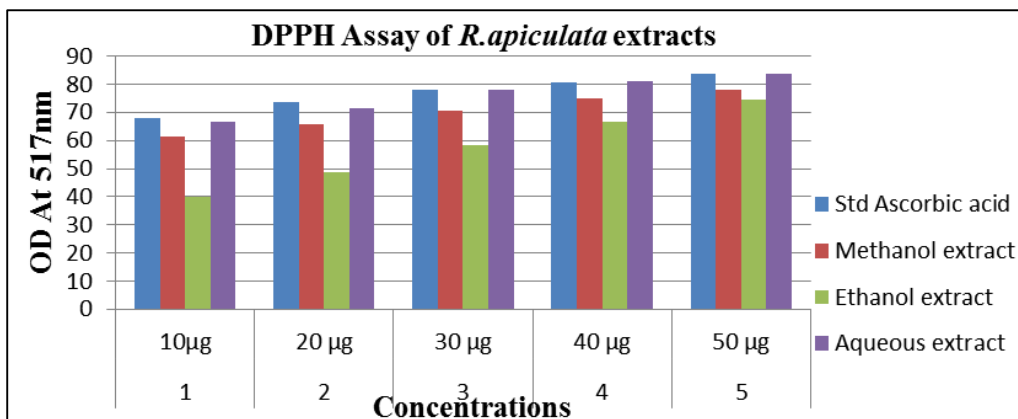


Fig 12: Percentage inhibition of DPPH free radical of *Rhizophora apiculata* extracts

## 5. Conclusion

Phytochemical analysis of *H. ponga*, *K. candel*, *V. leucoxydon* and *R. apiculata* plants showed that each extract contains broad spectrum of bioactive compounds that included alkaloids, glycosides, saponins, tannins, phenols and flavonoids. These bioactive compounds already reported to have several medicinal properties which include anticancer, anti-proliferative, antimicrobial, antioxidant, anti-tuberculosis, antimicrobial, anti-inflammatory, hemolytic agent and antiviral. The present study also showed high phenolics and flavonoids content in methanol extract of *K. candel* and aqueous extract of *V. leucoxydon* respectively. In context of antioxidant assays almost all selected extracts showed moderate activity but aqueous extract of *V. leucoxydon* and *R. apiculata* proven to be having significant antioxidant activity over all extracts. In case of anti-inflammatory activity, methanol extract of *K. candel* showed significant noticeable anti-inflammatory activity on comparing with standard drug and remaining extracts. Statistically the antioxidant assays were observed significant difference ( $P < 0.01$  and  $P < 0.05$ ) in each extracts. These differences in the activity may be due the variation in the chemical composition of extracts. Thus the present study concludes that aqueous extract of *V. leucoxydon* and *R. apiculata* and methanol extract of *K. candel* exhibited higher antioxidant and anti-inflammatory activities respectively. However further studies are needed to find out the structural of bioactive compounds and investigate mechanisms of antioxidant and anti-inflammatory activities of the bioactive compounds.

## 6. Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication

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