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## Antimicrobial appraisal and antioxidant potential of *Anisomeles indica* (L.) Kuntze (Lamiaceae)

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### Abstract

Plant extracts of *Anisomeles indica* prepared in different solvents were analyzed for their antimicrobial activities using disc diffusion assay against six bacterial and four fungal strains. The total phenolic and flavonoid content was also determined quantitatively. Out of the six plant extracts, methanolic extract showed the highest amount of phenols ( $153.7 \pm 0.75 \text{ mgGAE/g}$ ) and flavonoids ( $173.85 \pm .506 \text{ mg Quercetin/g}$ ) and it was positively correlated with antimicrobial potential of the extract. Free radical scavenging activity was evaluated by non-enzymatic assays (DPPH and ABTS assay) and enzymatic assays. Our results concluded that methanol extract exhibited highest antioxidant activity via DPPH and ABTS assay and also display high phenolic and flavonoid content. The enzymatic activity level of *A. indica* was found to be satisfactory.

**Keywords:** *Anisomeles indica*, phytochemicals, ABTS, DPPH, antimicrobial

### 1. Introduction

Plants are the source of bioactive compounds, used as herbal medicines since ancient times. Particularly in the developing countries from the hundreds of generations, the majority of population depends on medicinal plants to complete primary health care needs [1-5]. Synchronously, in developed countries, uses of medicinal herbs have been initiated as alternative or complementary of synthetic drugs, because of high resistivity of microbes against synthetic drugs. Phytochemicals are very significant to humans, not because of their therapeutic potential and also being devoid of side effects often associated with the synthetic drugs [6]. In India, most of these compounds broadly used in Ayurveda as medicine to cure disease. The global demand of herbal medicine is not so large, but it is also growing [5]. Current estimates suggest that not only medicinal plants, rather weeds also contained numerous phyto-constituents that are biologically active and used as medicine many parts of the world [7]. The weeds are unwanted plants that grow with the crops plants and also act as inhibitors for plants in respect of nutrients, space, water etc. But weeds also have phytochemicals that use in the pharmaceutical industries for the formulation of herbal drugs [8]. In many countries where in the rural areas, there is limited resources of modern health care, weeds used as medicine [9]. Weeds also showed effective antimicrobial, antioxidative, and anti-inflammatory properties [8].

*Anisomeles indica* (L.) Kuntze is member of *Lamiaceae/ Labiatae* family. It is commonly called as 'Indian Cat mint' and 'Kalabhanga' in India [10] and grows as weed. It has strong camphor-scented odour, erect herbaceous herb found throughout tropical and subtropical regions of Asia including India [10]. The plant is used to cure in gastric catarrh, bowels, increase thickness of intestinal mucus layer, infection of stomach and intermittent fever [11, 10]. The essential oil present in the herb is useful in uterine affections, [10, 12]. In the ancient time aerial parts were used to treat disorders such as paralysis, epilepsy, convulsions, spasm, pregnancy, rheumatism. Leaves of *A. indica* are chewed for toothache, paste used for chronic skin eruptions and also applied on snake bite [13, 14, 15]. It contains secondary metabolites such as flavonoids, diterpenoids, phenyl propanoids, steroids [16, 17]. The plant also showed activity against active *Helicobacter pylori* infection [14]. The aim of study to screen out the phytoconstituents of *A. indica* extracts of different solvent antioxidant activity and to evaluate the antimicrobial activity against different bacterial and fungal strains.

### 2. Material and Method

#### 2.1 Preparation of plant extracts

Fresh aerial vegetative parts of *A. indica* were collected from Samar Gopalpur village near Rohtak, Haryana, India in the month of April, 2017 and shade air dried for 6-7 days. The dried material was grinded to produce fine powder.

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Six different extracts were prepared in different solvents according to their polarity viz. methanol, ethanol, aqueous, acetone, and chloroform and petroleum ether by using Soxhlet apparatus. In Soxhlet extractor, 50grams of grinded powder was taken with 250ml of respective solvent in bottom flask. After 25-30 cycles, the solvent evaporated using rotary evaporator that leave small yield of extract. The extracts were stored at 4°C for further uses.

## 2.2 Phytochemical analysis

Phytochemical screenings were carried out for the detection of alkaloids, steroids, glycosides, Saponins, terpenoids, flavonoids, tannins and phenols for all five plant extracts prepared in different solvents. The methodology used for the qualitative analysis of different phytoconstituents is shown below:

**Alkaloids:** 2 ml of 1% HCL was added in 1ml of extract and heated gently for few minutes after it; 2-3 drops of Mayer's reagent <sup>[18]</sup> and Wagner's reagent <sup>[19]</sup> were added. Turbidity of precipitate appeared indicating the presence of alkaloids.

**Steroids:** 2ml of conc. H<sub>2</sub>SO<sub>4</sub> and 2ml of methanol were added in all different extracts with volume of 1ml. The appearance of layer of red color in the lower side of test tube indicated the presence steroid <sup>[20]</sup>.

**Glycosides: For glycosides two tests were performed:**

(1) **Salkowski test:** 2ml of chloroform was added in 1 ml of plant extract and few drops of conc. H<sub>2</sub>SO<sub>4</sub> were added from the side of test tube. A reddish brown colored ring on the test tube indicated the presence of glycosides <sup>[21]</sup>.

(2) **Kellar killani test:** Glacial acetic acid (2ml) was added along with 1-2 drops of 2% FeCl<sub>3</sub> solution were added in the 1ml of all different extracts. The mixture was poured in another test tube contained 2 ml of conc. H<sub>2</sub>SO<sub>4</sub>. A brown color ring appeared at interface showed the presence of glycosides <sup>[22]</sup>.

**Saponins:** In a different test tubes, 5 ml of ds H<sub>2</sub>O were mixed with, 1ml of extracts and shaken vigourously. A stable soap like foam indicated the presence of Saponins <sup>[23]</sup>.

**Terpenoids:** 2 ml of methanol was mixed with 1ml of extracts and evaporated to dry. After few minutes, in the test tube 3 ml of conc. H<sub>2</sub>SO<sub>4</sub> added. A reddish brown color appeared at interface <sup>[24]</sup>.

**Flavonoids:** By alkaline reagent test <sup>[22]</sup>, 2-3ml of dil. NaOH was added in 1ml of extract. A yellow color appeared followed by addition of 3-4 ml dilute HCl, turn yellow color to colorless showed the presence of Flavonoids.

**Phenols: Ferric Chloride test:** 2-3 drops of ferric chloride was added directly in plant extracts. Appearance of violet-blue color indicated the presence of phenols <sup>[25]</sup>.

**Tannin:** Bramer's test: 2ml ds H<sub>2</sub>O was added in 2ml of extract and that mixture was treated with 2 to 3 drops of 10% ferric chloride. Presence of tannin indicated as blue-green color <sup>[22]</sup>.

## 2.3 Total phenolic content

Estimation of phenolic content was done by Folin-Ciocalteu reagent method as followed up <sup>[26]</sup>. In the alkaline medium,

phenol was reacted with phosphomolybdic acid of Folin-Ciocalteu reagent that produced molybdenum blue that was blue in color, which was determined spectrophotometrically at 650 nm. A stock solution of plant extracts was prepared to 1mg/ml. From stock solution, 1ml of plant extract was added in 5ml of 10% v/v Folin-Ciocalteu and 2ml of Na<sub>2</sub>CO<sub>3</sub>. The mixture was vortexed for 2minutes and incubated in dark for 15minutes. The absorbance was measured at 650nm. Blank solution consisted of 5ml Folin-Ciocalteu, 1ml of solvent and 2ml of Na<sub>2</sub>CO<sub>3</sub> solution. Gallic acid was used as standard of 10ug/ml-100ug/ml range from stock of 1mg/ml. By using calibration curve, the unknown concentration of samples was obtained and results were expressed in terms of milligrams of Gallic acid equivalent (mg of GAE)/gram dry weight (g DW). All tests were performed in triplicates. Total phenolic content was calculated using following equation:

$$\text{Total phenolic content} = C \times \frac{V}{m} \quad \text{Eq. 01}$$

Where 'V' is the volume of extract in ml, 'C' is the concentration (mg/ml) and 'm' is the weight (g) of plant extract.

## 2.4 Total flavonoid content

Flavonoid content was estimated by using aluminum chloride method spectrophotometrically with some modifications [26]. 1ml of plant extract was enumerated with 3ml of methanol, 0.2ml of (10% w/v) aluminum chloride and 0.2ml of 1M sodium acetate. The total volume of mixture was raised to 10ml by using distilled water. The samples were leave at room temperature for 30 minutes and then estimated spectrophotometrically at 420nm. Quercetin was used as standard with different concentration (10ug/ml-100ug/ml). All the measurement was carried out in triplicates. The unknown concentrations were found out by calibration curve and results were demonstrated as Quercetin equivalent (mg/g of extracted compound). Total flavonoid content was calculated by using equation 02:

$$\text{Total flavonoid content} = C \times \frac{V}{m} \quad \text{Eq. 02}$$

Where V is the volume of extract in ml, C is the concentration (mg/ml), and m is the weight (g) of plant extract.

## 2.5 DPPH assay

The free radical scavenging activity of different extracts was measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay <sup>[27]</sup>. To 2.5ml of different concentrations (10µg/ml to 100µg/ml) of sample, 1ml of 0.3mM DPPH solution was added and incubated in dark for 30 minutes. Ascorbic acid was used as standard and DPPH solution without extract was used as control. The decrease in absorbance of sample from 10µg/ml to 100µg/ml was estimated spectrophotometrically at 517nm. All measurements were carried out in triplicates. The absorbance was converted into percentage antioxidant activity by using following equation 03:

$$\text{Free radical scavenging activity (\%)} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100 \dots \text{Eq. 03}$$

## 2.6 ABTS assay

ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay was carried out as per the slightly modified method of Shirwaikar *et al*, 2006 <sup>[28]</sup>. ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution

with 2.45 mM ammonium per sulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS solution was diluted with ethanol to get an absorbance of 0.700 at 745 nm. A stock solution of plant extract was prepared to 1mg/ml in their respective solvent. Each stock solution diluted from 10- 100 µg/ml. One ml solution of ABTS was added to 1ml of sample solution of different concentration. Ascorbic acid was used as standard with same concentration (10-100µg/ml). The absorbance was measured at 745nm by UV-Vis spectrophotometer (SHIMADZU 1800, Japan). Antioxidant activity was calculated with the help of following equation-4.

$$\text{Free radical scavenging activity (\%)} = \frac{[\text{Abs (control)} - \text{Abs (sample)}] \times 100}{\text{EQ-4}}$$

## 2.7. Extract for enzymatic Assays

From the freshly collected plant sample (0.5gm), the plant extract was prepared by homogenization in 5ml of cold extraction buffer containing 100mM potassium phosphate buffer (pH7.0) and 0.1mM sodium ethylene diamine tetraacetic acid (EDTA). The homogenized extract was centrifuged at 14000 rpm for 20 minute at 4°C and supernatant was directly used for all the enzymatic assays.

### 2.7.1 Catalase assay

Catalase activity was estimated by the method of Aebi, 1984 [29]. Total 3ml reaction mixture contained 100µL of plant extract, 10 mM H<sub>2</sub>O<sub>2</sub>, and 50 mM (pH-7.0) potassium phosphate buffer. Absorbance was measured at 240 nm. In every 30 sec, for 5minutes there is decrease in absorbance. Activity of plant extract enzyme was expressed as µmoles of H<sub>2</sub>O<sub>2</sub> decomposed mg<sup>-1</sup> fresh weight min<sup>-1</sup>. One unit of catalase will decompose 1.0 µmole of H<sub>2</sub>O<sub>2</sub> per minute at pH 7.0 at 25 °C under the assay condition. (EC of H<sub>2</sub>O<sub>2</sub> is 39.4 mM<sup>-1</sup>cm<sup>-1</sup>.)

### 2.7.2 SOD assay

Superoxide dismutase (SOD) activity was determined by Misra *et al.* 1972 [30]. 3ml reaction mixture contained 100µl plant extract, 75 mM NBT, 50mM potassium phosphate buffer, 13 mM methionine, 2µM riboflavin and 0.1 mM EDTA. After shaking of reaction mixture, the tubes were placed in the light intensity of 5000 lux for 25minutes. The absorbance was measured at 560 nm. Reaction mixture placed in dark did not produce any colour, served as the control. One unit activity was measured by amount of the enzyme required to causes 50% inhibition of the reduction of NBT per unit time. That was measured at 560 nm.

### 2.7.3 GST assay

Glutathione S-Transferase activity was estimated by Habig *et al.*, 1974 [31]. 3 ml reaction mixture of 97mM potassium phosphate buffer (pH-6.5), 1mM EDTA(in DW), 30mM CDNB (prepared in 95% ethanol) from 4°C and GSH 75 mM (in Buffer) from -20°C freezer were taken. Absorbance was measured at 340 nm for every 30 upto 5 minutes. One unit of activity was calculated by the amount of enzyme which produces 1.0 µmol of GS-DNB conjugate/min under the conditions of the assay. (EC of GS-DNB conjugate at 340 nm is 9.6 mM<sup>-1</sup>cm<sup>-1</sup>).

## 2.8 IC<sub>50</sub> value

IC<sub>50</sub> value (mg/ml) was determined by plotting graph of concentration against percentage inhibition which is defined

as amount of plant extract having antioxidants quench 50% free radicals of DPPH.

### 2.8.1 In vitro antimicrobial evaluation of *A. indica*

The antimicrobial activities of extracts of *A. indica* were assessed against three gram negative: *Klebsiella pneumonia* (MTCC109), *Pseudomonas aeruginosa* (MTCC2453), *Escherichia coli* (MTCC40) and three gram positive: *Staphylococcus aureus* (MTCC96), *Bacillus subtilis* (MTCC2057) and *Mycobacterium smegmatis* (MTCC992). The bacterial strains were procured from IMTech, Chandigarh, India. The bacterial strains were cultured in nutrient broth and incubated at 37°C overnight. Suspension of the bacterial strains was further diluted with sterile peptone water and checked the turbidity according to Mcfarland standard until 10<sup>8</sup> CFU/mL (turbidity = McFarland barium sulfate standard 0.5) [32] and the absorbance was obtained at 600nm. Potential of the plant extracts also assessed against four fungal strains namely *Aspergillus niger* (MTCC514), *Fusarium oxysporum* (MTCC7392), *Rhizopus oryzae* (MTCC-262) and *Penicillium expansum* (MTCC2818).

### 2.8.2 DISC diffusion assay

The antimicrobial potential of plant extract was determined by Disc diffusion assay (CLSI, 2015) [33]. The sterile disc of 6mm diameter of what man paper 1 were soaked with 15µl of plant extracts having various concentrations(100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml). The discs of different concentration were placed on agar plates, dried and incubated at 37°C overnight for 18 hours. Ampicillin (100µg/ml) (Hi-Media Laboratories Pvt. Ltd. India) was taken as positive control and the different solvents (used for preparation of plants extract) were expressed as negative control. After incubation, evaluation was done by measurement of the diameter of the inhibited zone (IZ) in mm using a plastic ruler. All measurements were repeated thrice and the mean was taken by statistical analysis and standard deviation in MS Excel program.

Antifungal activity was also determined by disc diffusion assay [33, 34] as described above. The fungal culture was grown on Czapek dox broth (Hi-media) for *A. niger* and potato dextrose broth (Himedia) for *F. oxysporum* and *P. expansum*. Inoculums were applied on the surface of the Czapek's dox agar and Potato Dextrose agar plates and spread out. The sterile disc (6mm diameter) was soaked with different concentration of extracts (200ug/ml, 100ug/ml and 50ug/ml) and incubated for 48 hrs at 28°C. Miconazol (Hi-Media Laboratories Pvt. Ltd. India) antibiotic was used as positive control and different solvents as negative control.

### 2.8.3 Determination of MIC

The MIC of *A. indica* was determined by using micro dilution assay [35]. MIC of different plant extracts was evaluated against six bacterial strains in 96 well microtiter plates with addition of 100 µL of nutrient broth in each well. 100 µL plant extract were serially diluted in each well and 10 µL of test organism suspension was added to each well containing 10 µL of rezaurin dye. MIC plates were incubated at 37°C for 24hrs. The lowest extract concentration that inhibited the test organism was recorded as MIC. For antifungal activity, Czapek's dox broth was used for *A. niger* and potato dextrose broth was used for *F. Oxysporum*, *P. expansum* and *R. oryzae*. The incubation of plates at 28°C for 48 hrs and the inhibitions of fungal strains were directly visualized without any coloring agent.

### Statistical analysis

All graphs were created and statistical calculations were carried out using Microsoft Excel. Correlations between the antioxidant activity and total phenolic content were examined using Pearson's correlation. All the experiments were performed in triplicates and expressed as average  $\pm$  standard deviation.

## 3. Result and Discussion

### 3.1 Phytochemical screening

Qualitative phytochemical analysis of plant extracts of *A. indica* indicated the presence of phenolic and flavonoid content in all extracts and the result obtained are given in

**Table 1:** Presence (+), and absence (-) of different phytochemicals of *A. indica*

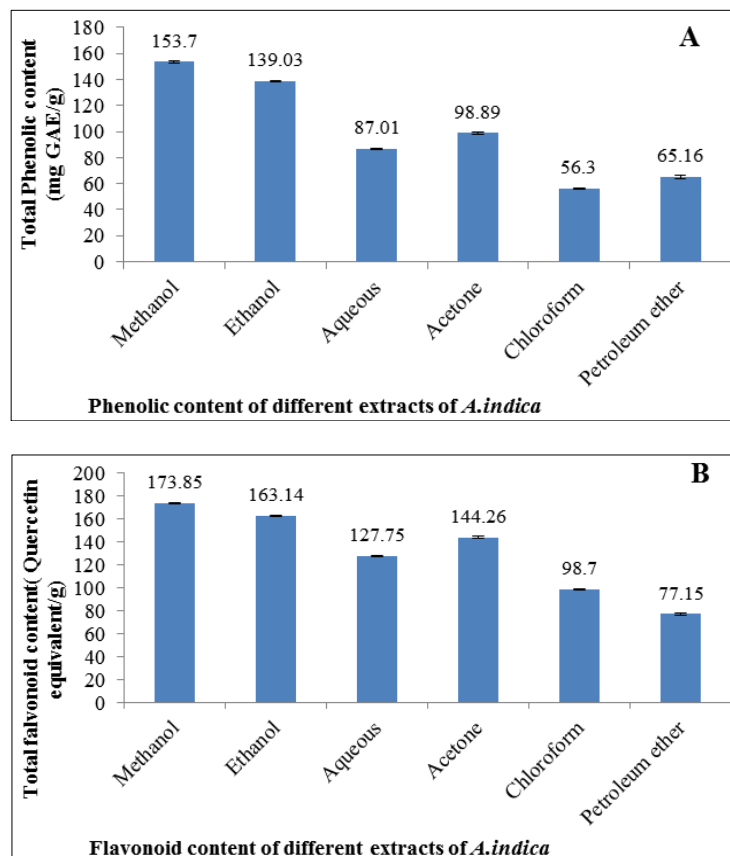
Solvent	Steroid	Terpenoid	Alkaloid	Saponins	Glycosides	Tannins	Phenols	Flavonoid
Methanol	+	-	+	-	+	+	+	+
Ethanol	+	-	+	-	+	+	+	+
Aqueous	+	-	-	-	+	-	+	+
Acetone	-	-	+	-	+	+	+	+
Chloroform	-	+	-	+	-	-	+	+
Petroleum ether	-	+	-	+	-	-	+	+

### 3.2 Total phenolic and flavonoid contents

The results showed the amount of total phenolic and flavonoid contents differed significantly among the extracts of the *A. indica* [Figure 2a, 2b]. The total phenolic contents were determined as mgGAE/g extract in comparison with standard gallic acid graph. Two plants extract methanolic (153.7 $\pm$ 0.75mgGAE/g) and ethanolic extract (139.03 $\pm$  0.34 mg GAE/g), aqueous (87.01 $\pm$  0.34 mg GAE/g), acetone(98.84 $\pm$  0.88 mg GAE/g), chloroform(56.30 $\pm$  0.56 mg GAE/g) and petroleum ether(65.16 $\pm$  0.93 mg GAE/g) based extracts showed low phenolic content respectively. The total flavonoid content was determined as Quercetin mg/g of

table1. The methanolic and ethanolic extracts showed the presence of steroids, alkaloids, glycosides and tannins except terpenoids and tannins. However the acetonic extracts showed the presence of all other phytochemicals except steroids, terpenoids and saponins. Other three plants extract aqueous, chloroform and petroleum ether showed presence of terpenoids, saponins, phenols, and flavonoids. Phytochemicals such as flavonoids, phenols, tannins and terpenoids has biotherapeutic effects like anti-inflammatory, anti-diarrhea [36, 3] *A. indica* plant extracts specifically methanol and ethanol extract showed almost all these phytochemicals that may be indicate the potency of antimicrobial activity.

extract after comparison with a quercetin standard graph. The highest amount of flavonoid content was found to be in methanolic (173.85 $\pm$ .506 mg Quercetin/g) followed by ethanolic extract (163.14 $\pm$ 0.41 mg Quercetin/g). However acetonic extract (144.26 $\pm$ .81 mg Quercetin/g) showed highest after ethanolic extract followed by aqueous extract (127.5 $\pm$ 0.36mg Quercetin/g), chloroform extract (98.70 $\pm$ 0.66 mg Quercetin/g) respectively. Lowest amount of flavonoid content was obtained in petroleum ether (77.15 $\pm$ .784mg Quercetin/g). Flavonoid and phenols are largest group of compound found ubiquitously in plants and competent for antioxidant property [3].



**Fig 2A, 2B:** Total phenolic and flavonoid content of all extract of *A. indica*

### 3.3 DPPH and ABTS radical scavenging activity

The radical scavenging activities were evaluated by DPPH and ABTS assay by percentage inhibition. By antioxidative properties of plant extracts, DPPH and ABTS radicals were showed to decolorize. Our results demonstrating strong ability of methanolic extract to decolorize the radicals of DPPH and ABTS followed by ethanolic, acetonic, chloroform extracts that were represented in Figure 3a, 4a. The petroleum ether and aqueous extract both showed the lowest activity. The results of different plant extracts showed with relativity of standard Ascorbic acid. Similarly the radical scavenging also detected by ABTS assay. All six plant extracts exhibited to neutralize the ABTS radicals. In previous study, the antioxidant property of the different extracts of *A. indica* was revealed that DPPH and ABTS assay scavenge free radicals in dose dependent manners and also depend on polarity of extract [39, 40, 41, 42]. Our study indicated notable oxidative property it may be due to presence of high amount of phenolic and flavonoid content.

### 3.4 IC<sub>50</sub> value of extracts

IC<sub>50</sub> value (µg/ml) was determined by plotting graph of percentage inhibition against six plant extracts concentration (shown in Figure 3b, 4b). A low IC<sub>50</sub> value shows high antioxidant activity. In case of DPPH assay, methanolic extract showed the least value of IC<sub>50</sub> (69.38µg/ml) hence highest antioxidant activity followed by the ethanolic extract (86.922µg/ml), acetonic extract (96.32µg/ml), petroleum ether (146.84 µg/ml), aqueous (154.02 µg/ml) least activity was shown by Chloroform extract having highest IC<sub>50</sub> value (158.15µg/ml). Similarly as DPPH assay, in the ABTS assay, methanolic extract showed the least value of IC<sub>50</sub> (43.05µg/ml) followed by the ethanolic extract (57.13µg/ml), acetonic extract (71.22µg/ml), chloroform (77.97 µg/ml), aqueous (102.51 µg/ml) and least antioxidant activity was shown by petroleum ether extract having highest IC 50 value (106.9µg/ml).

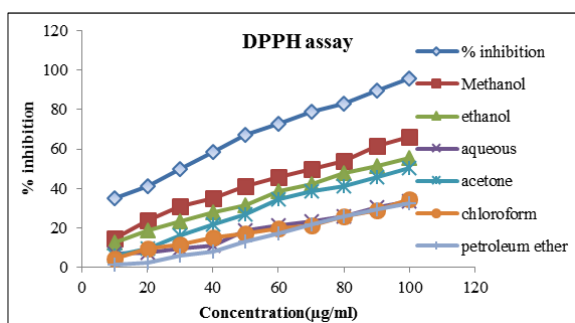


Fig 3a: Percentage inhibition of DPPH radical by different plant extracts

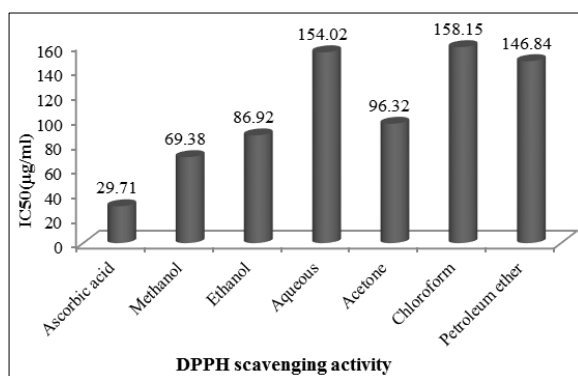


Fig 3b: IC<sub>50</sub> values of plant extracts

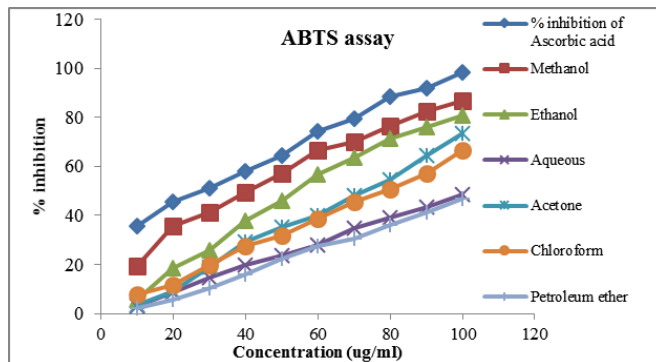


Fig 4a: Percentage inhibition of ABTS radical by different plant extracts

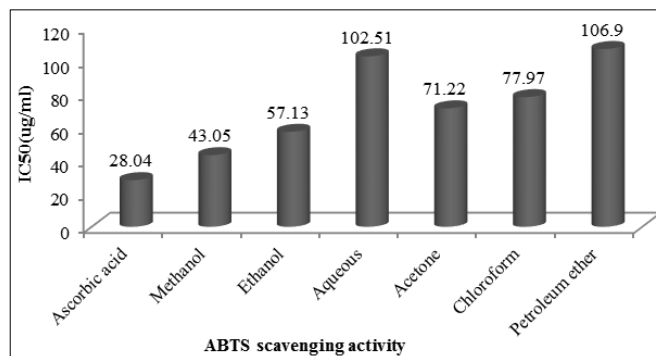


Fig 4b: IC<sub>50</sub> values of plant extracts

### Correlation between IC<sub>50</sub>, Total phenolic and flavonoid content

It is clearly indicated that correlation between TPC/TFC and IC<sub>50</sub> values are negative, which represent that plant extracts have high TPC/TFC content and lower IC<sub>50</sub> values. Our finding represented that, TPC have more contribution for the antioxidative potential than the TFC as shown good correlation ( $R > 0.9$ ). A good correlation was observed between TPC and antioxidant potential of the plant extracts in our study which is in conformity with other studies [43].

Table 2: Correlation between Antioxidant activities (IC<sub>50</sub> Values), Total phenolic and flavonoid contents

Pearson correlation coefficient (r)				
TPC and TFC	TPC and values of ABTS	TFC and IC <sub>50</sub> values of ABTS	TPC and IC <sub>50</sub> of DPPH	TFC and IC <sub>50</sub> values of DPPH
0.936	-0.817	-0.833	-0.922	-0.872

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

### 3.5 Enzymatic assays

Catalase, GST and Superoxide dismutase enzymes are potent indicator of antioxidant activity in plants, involved in defense system [44]. The activity results obtained in case of antioxidant enzymes namely, Catalase, Glutathione S-transferase and Superoxide Dismutase shown in Table 3. The enzyme activity was found to be  $0.633 \pm 0.021$  µmole of H<sub>2</sub>O<sub>2</sub> per minute FW for Catalase,  $34.44 \pm 0.60$  µmol of GS-DNB conjugate/min FW for Glutathione-S- Transferase and  $19.12 \pm 0.21$  SOD (Unit) FW for Superoxide dismutase respectively.

Table 3: Antioxidant enzyme activities of *A. indica*

Antioxidative enzyme	Activity
Catalase	$0.633 \pm 0.021$
Glutathione-S- Transferase	$34.44 \pm 0.60$
Superoxide dismutase	$19.12 \pm 0.21$

Values of enzymatic assays are represented as mean (n=3) ±SD.

Enzyme activity unit represented as:

**CAT-** One unit of catalase decompose 1.0 μmole of H<sub>2</sub>O<sub>2</sub>/minute at pH 7.0 at 25 °C.

**SOD-** One unit of SOD activity is the amount of enzyme required to cause 50% of inhibition of NBT/ unit time that was measured at 560 nm.

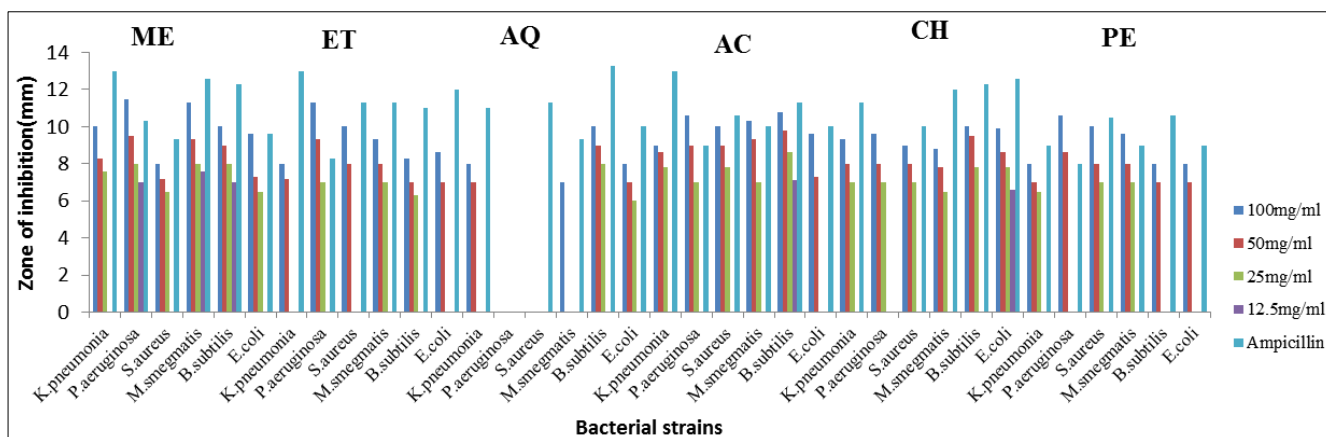
**GST-**One unit of GST activity is the amount of enzyme, produces 1.0 μmol of GS-DNB conjugate/min.

**3.6 Antimicrobial activity of A. indica**

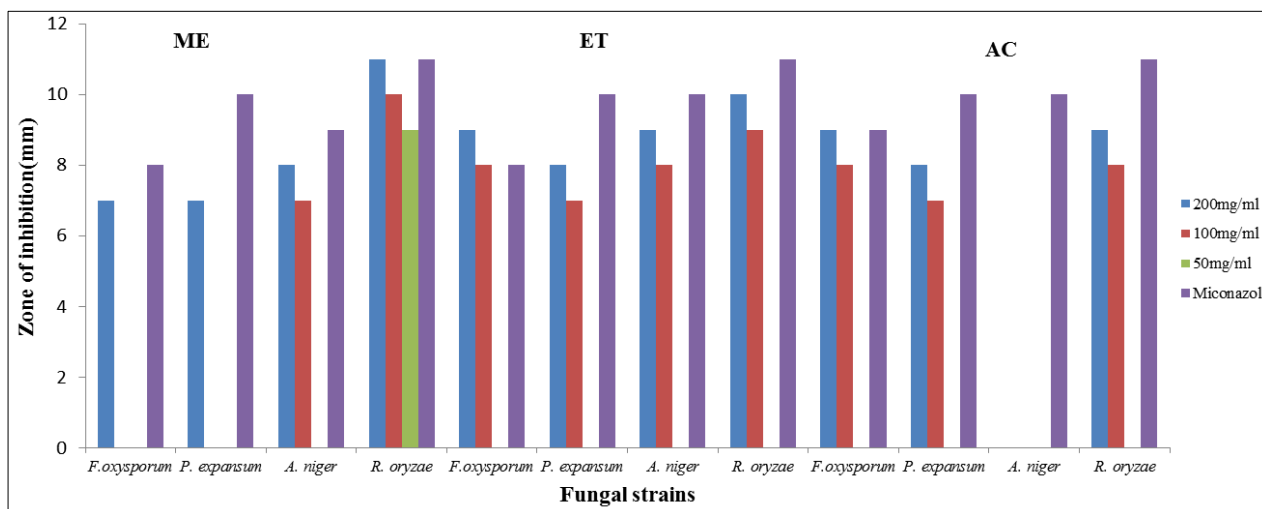
The antimicrobial activity of six plant extracts of *A. indica* were assessed against six bacterial strains and four fungal strains by disc diffusion method and obtained result represented graphically in figure: 5, 6. The plant extract prepared in methanol solvent showed maximum zone of inhibition against four bacteria *P.aeruginosa* (11.5±0.50), followed by *M. smegmatis* (11.3±0.57), *K. pneumonia* (10±0) and *E. coli* (9.6±0.5). The ethanolic extract showed highest inhibition against *P.aeruginosa* (11.3±0.57) followed by *S. aureus*(10±0), *E. coli* (8.6±0.57), *B. subtilis* (8.3±0.57), *K. pneumonia* (8±0). The acetic extract showed highest inhibition against *B. subtilis* (10.8±0.56), *P. aeruginosa* (10.6±0.56), *M. smegmatis* (10.3±0.57), *S. aureus* (10±0), *E. coli* (9.6±0.57), *K. pneumonia* (9±0). The aqueous extract

showed highest zone of inhibition against *B. subtilis* (10±0) followed by *E. coli* (8±0) = *K. pneumonia* (8±0). The aqueous extract is not working against *P. aeruginosa*, *S. aureus* and *M. smegmatis*. However chloroform extract showed maximum inhibition against *B. subtilis* (10±0). The petroleum ether extract is very least effective against all bacteria. The collective analysis of antimicrobial activity of *A. indica* indicated range of zone of inhibition from 8 mm to 11.5 mm on against different six bacteria. Maximum ZOI recorded with the positive controls that were Ampicillin with highest ZOI 12.6±0mm against *M. smegmatis*.

On the other hand all the extracts of *A. indica* were not so much effective against fungal strains as compared to bacterial strains. Although methanolic extract showed highest activity against *R. oryzae* (11±0) that were equal to the standard antibiotic miconazol (11±0). The ethanolic extract showed highest activity against *A. niger* (9±0) that was lower than miconazol (10±0) and *F. oxysporum* (9±0) that was equal to positive control (9±0). The ethanolic and acetic extracts both showed same activity against *P. expansum* (8±0). The previous studies showed resistivity of plant against fungal strains [45-47]. According to previous studies, the activity against both kinds of gram positive and gram negative bacteria indicated that extract is having more bioactive compounds [48].



**Fig 5:** Disc diffusion assay of six different *A. indica* extracts, ME- Methanol, ET-Ethanol, AQ- aqueous, AC-Acetone, CH-Chloroform, PE- Petroleum ether, The zone of inhibition showed as mean ±standard deviation (n=3), *K. pneumonia*: *Klebsiella pneumonia*, *P.aeruginosa*: *Pseudomonas aeruginosa*, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *M. smegmatis*: *Mycobacterium smegmatis*, *B. subtilis*: *Bacillus subtilis*



**Fig 6:** Disc diffusion assay of three *A. indica* extracts, ME- Methanol, ET-Ethanol, AC-Acetone, *F. oxysporum*: *Fusarium oxysporum*, *P. expansum*: *Penicillium expansum*, *A. niger*: *Aspergillus niger*, *R. oryzae*: *Rhizopus oryzae*.

### 3.7 MICs value

MICs value of plant extracts against bacterial and fungal strains was shown in table 4, 5. The methanolic extract showed lowest value against *M. smegmatis* (0.39µg/ml), followed by *B. subtilis* (1.5639µg/ml), *E. coli* (1.5639µg/ml), *P. aeruginosa* (3.125µg/ml), *S. aureus* (3.125µg/ml), *P. aeruginosa* (6.25µg/ml). The ethanolic extract also showed lowest activity against *M. smegmatis* (0.78µg/ml). Other plant extracts such as acetone, chloroform and petroleum ether displayed almost same range of MICs value from 3.125µg/ml to 6.25µg/ml. On the other hand aqueous extract showed higher MICs that were very less effective against all bacteria.

In case of four fungal strains where methanolic extract showed lowest MICs values against *A. niger* (1.56µg/ml) followed by *R. oryzae* (6.25µg/ml). Similarly ethanolic extract showed lowest value against *A. niger* (6.25µg/ml) followed by *R. oryzae* (6.25µg/ml). The other extracts such as acetone, chloroform and petroleum ether showed same range of MICs from 12.5 µg/ml to 50µg/ml. The aqueous extract was not shown any MICs against all fungal strains. Although miconazol showed very low MICs value against all three fungal strains ranged from 0.39µg/ml to 1.56µg/ml.

**Table 4:** MIC values (ug/ml) of different extracts of *A. indica* against different bacterial strains, Unit: µg/ml

MICs value of <i>A. indica</i>							
Bacterial strain	Methanol	Ethanol	Aqueous	Acetone	Chloroform	Petroleum ether	Ampicillin
<i>K. pneumonia</i>	6.25	12.5	50	12.5	25	25	0.097
<i>P. aeruginosa</i>	3.125	12.5	25	12.5	25	25	0.19
<i>E. coli</i>	1.56	3.125	50	6.25	12.5	12.5	0.19
<i>S. aureus</i>	3.125	6.25	100	3.125	6.25	12.5	0.39
<i>M. smegmatis</i>	0.39	0.78	50	1.56	6.25	6.25	0.19
<i>B. subtilis</i>	1.56	3.125	50	1.56	3.125	6.25	0.097

The MIC values showed as mean d (n=3).

**Table 5:** MIC values (mg) of different extracts of *A. indica* against different fungal strains

MICs value of <i>A. indica</i>							
Fungal strain	Methanol	Ethanol	Aqueous	Acetone	Chloroform	Petroleum ether	Miconazol
<i>F. oxysporum</i>	12.5	25	0	50	25	25	0.78
<i>P. expansum</i>	25	50	0	12.5	0	6.25	1.56
<i>A. niger</i>	1.56	6.25	0	12.5	0	0	0.78
<i>R. oryzae</i>	6.25	6.25	0	12.5	0	0	0.39

The MIC values are showed as mean (n=3)

### 4. Conclusion

This study showed antibacterial activity of six different extracts of *A. indica* against six different bacterial and four fungal strains by using disc diffusion and MICs assay. Methanolic extract showed highest antibacterial activity against all the pathogens. Free radical scavenging activity was evaluated by using DPPH and ABTS assay and SOD, Catalase and GST assays have significant values. All other extracts showed moderate antimicrobial, antioxidant and phytochemical activity. Besides, methanolic extract showed the highest amount of phenolic (153.7±0.75mgGAE/g), and flavonoid (173.85±.506 mg Quercetin/gm) contents after quantitative observation. The methanolic extract also showed highest antioxidant activity with IC<sub>50</sub> value 69.38 µg/ml of DPPH assay and 43.05 µg/ml of ABTS assay. Our study suggested that methanolic extract will be used to isolate bioactive compound and can be assessed for their therapeutic potential.

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