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Purification and characterization of bioactive compounds extracted from (Hoom) *Miliusa tomentosa*

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Abstract
Introduction: Green plant possesses highly diversified list of natural phytochemicals which are known to have wide range of bioactivities. These phytochemicals can be good sources for the discovery of new drugs. In this study, we report the partial phytochemical characterization and antimicrobial activities of a semi-purified fraction isolated from the *Miliusa tomentosa* plant belongs to family Annonaceae.
Materials: The plant was taxonomically identified and leaves were collected at Umbalebailu, Bhadra Wildlife Sanctuary, Shivamogga, Karnataka, India.
Methods: Extraction of bioactive molecules by using Chloroform for 10 hours by magnetic stirrer. The solvent extracted sample was subjected to phytochemicals analysis followed by purification by column chromatography. The separated fractions were confirmed by TLC and purified fractions were elevated for anti-oxidant and antimicrobial activities.
Results: All the three fractions have sugars and flavonoids, However, Fraction-3 has glycosides and terpenoids and other phytochemicals. Column purified fractions were exhibited significant amount of antioxidant and antibacterial activity.
Conclusion: The present findings suggest that the column purified fraction possesses effective antioxidant antimicrobial activities. Further studies are required to unveil its potential for application in pharmaceutical as well as food processing industries.

Keywords: Purification, food processing industries, characterization, natural phytochemicals

Introduction
Green plant possesses highly diversified list of natural phytochemicals which are known to have wide range of bioactivities. These phytochemicals can be good sources for the discovery of new drugs. Magnoliales and its family circumscription is clear and undisputed. The plants belonging to family Annonaceae are used as antibacterial, anticancer, anthelmintic, antiparasitic and pesticidal agents. The genus Miliusa (Annonaceae family) consists about 40 species which grows in tropical rainforest of India (Jumana *et al*., 2000) [1]. The plant is used in folk medicine for different symptom such as gastropathy and glomerulonephropathy. In Chinese traditional medicine Miliusa tomentosa oil has been found to have both antibacterial and analgesic properties (Kamerdick *et al*., 2002 and Huoung *et al*., 2008) [5, 6]. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances. Thus, main objective of this research work is to consider the phytochemical screening and their biochemical evaluation.

Methods
Preparation of plant extracts in organic solvent; 50 gm of dried leaf powder was subjected to magnetic stirrer extraction with 1000 ml of chloroform in a conical flask and stirred room temperature for 10 hours and filtered through Whatman No.1 filter paper. The concentrated extracts were taken in colour amber bottles and kept in refrigerator for further analysis using the standard methods.

Purification and partial characterization of *Miliusa tomentosa* leaf extract column chromatography
Active leaf extracts of *Miliusa tomentosa* was subjected to column chromatography on silica gel of 100–200 mesh size (Merck) packed in a cylindrical glass column. Sample was passed in the pre-packed column and eluted with mixture of hexane: Ethyl acetate as mobile phase to vary polarity to separate and collect fractions.

Extract was fractionated via gradient separation of mobile phase, and each elute was carefully collected and labelled for further analysis. All the fractions were pooled based on similarity in color and tested for antibacterial and antioxidant activity. Based on activity specific fractions were further purified using preparative TLC.

Antibacterial activity

Antibacterial activity of all the three extracts against *Bacillus subtilis* (MTCC 441) and *E. coli*, (MTCC 739) was determined through agar well diffusion method.

Antioxidant activity

Conversely, the antioxidant activity of *Miliusa tomentosa* leaf extract was determined using DPPH radical scavenging assay and FRAP assay.

Results and Discussion

Presence of glycosides, cardiac glycosides and reducing sugars was observed in crude and partial purified fractions. Column separated fractions were successful in inhibiting the growth of tested bacteria and values were very close to the activity of synthetic antibiotic ampicillin. The antioxidant activities for purified fractions at different concentrations were studied through DPPH radical scavenging and FRAP assay. In DPPH radical scavenging assay, the fractions were successful in donating protons and scavenging DPPH radicals. On the other hand, the FRAP assay was conducted to determine the electron transfer ability of *Miliusa tomentosa* leaves and found their successful reduction of Fe^{3+} to Fe^{2+} by measuring the absorbance at 593 nm which confirms the antioxidant activity. Reducing potential was expressed in ascorbic acid equivalents µg/mL and gradually increased with increase in concentration. Based on the present study, column purified fractions-3 was found comparatively potent than other fractions at 500 µg/mL concentration with an AAE value of 113 µg/mL, respectively (Ali *et al*., 2014) [7].

Table 1: Phytochemical screening of leaf extracts of *Miliusa tomentosa*

Phytochemicals	Crude	Fraction 1	Fraction 2	Fraction 3
Tannins	–	–	-	+
Saponins	–	–	-	–
Flavonoids	–	+	+	+
Glycosides	+	+	-	+
Terpenoids	+	+	-	+
Steroids	-	+	+	-
Phenols	–	+	+	+
Proteins	–	–	–	-
Phytosterols	+	+	+	+
Alkaloids	–	–	+	+
Cardiac glycosides	+	+	-	+
Reducing sugars	+	+	+	+

Table 2: Showing zone of inhibition of chloroform leaf extract of column purified fractions in cm

Fractions		Antibiotics	10	20	Solvent
1	*E coli*	2	2	1	0
	Bacillus subtilus	1	0.6	0.8	0
2	*E coli*	1	0.4	0.3	0
	Bacillus subtilus	0.6	0.2	0.3	0
3	*E coli*	1.3	0.7	0.6	0
	Bacillus subtilus	0	0.6	0.3	0

Fig 1: Column purification of *Miliusa tomentosa* chloroform leaf extracts

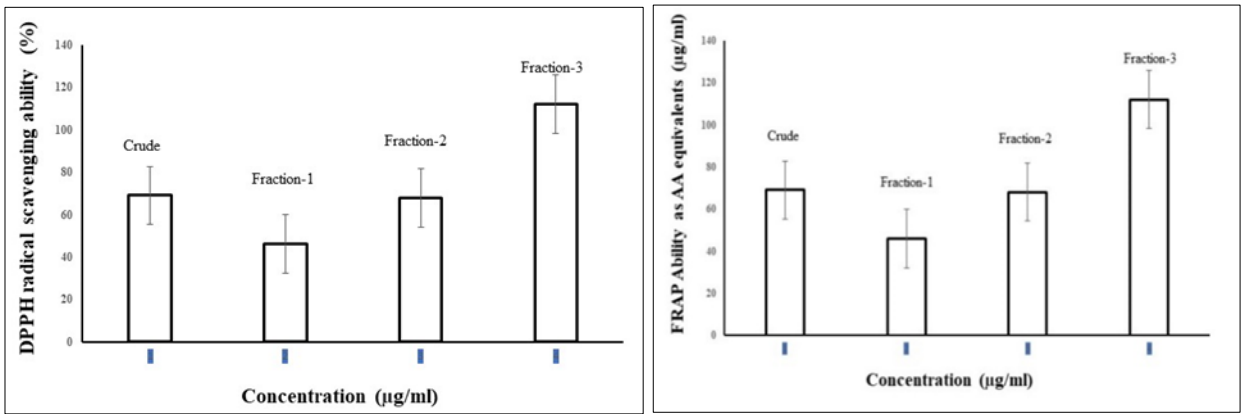

Fig 2: DPPH radical scavenging activity (a) and Ferric reducing antioxidant power ability (b) of *Miliusa tomentosa* leaf extracts

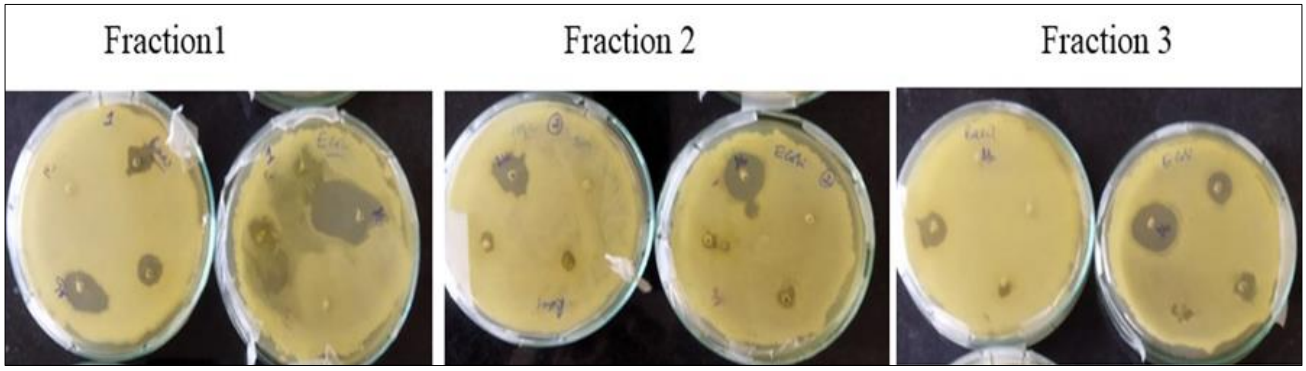

Fig 3: Antibacterial activities of partially purified *Miliusa tomentosa* leaf extracts

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Conclusions
The phytochemical screening of Miliusa tomentosa leaf extracts revealed the presence of various bioactive compounds including glycosides, flavonoids, terpenoids, and phenols. Column purification resulted in fractions with significant antibacterial and antioxidant activities, comparable to synthetic antibiotics and ascorbic acid. Fraction-3 exhibited the highest antioxidant potential. These findings suggest the therapeutic potential of Miliusa tomentosa in treating bacterial infections and oxidative stress-related conditions. Further studies are warranted to elucidate the mechanisms underlying its bioactivities and to develop therapeutic interventions harnessing its phytochemical constituents.

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