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Antibacterial Activity and Phytochemical Analysis of Methanolic and Acetonic Extracts from *Moringa oleifera*, *Vitex negundo* and *Rosa indica*

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A B S T R A C T

Throughout the ages, plants have been a valuable resource of natural products for human health. All parts of the plant, from root to fruit, consisting of a multitude of secondary metabolites which impart an unprecedented variety of medicinal uses to the plant. Studies have shown the presence of different phytochemical constituents in botanical sample responsible for the antimicrobial activity. These antimicrobial agents should be beneficial to host cells and toxic to pathogenic microbes. Some medicinal plants and spices have been reported to exhibit antibacterial activity. Hence, the antibacterial activity was examined from the leaf of *Moringa oleifera* and *Vitex negundo* and petals of *Rosa indica* through agar disc diffusion method. Each sample was collected and its crude extract was obtained by using methanol and acetone as the extraction solvent. These extract were tested against two Gram positive (*Bacillus cereus* and *Staphylococcus aureus*) and one Gram negative bacteria (*Salmonella typhi*). The methanolic and acetonic extract of each *M.oleifera*, *V.negundo* and *R. indica* showed antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhi*. Bio-chemical test for the presence of phytochemicals have shown positive result for tannin, flavonoid and alkaloid. These phytochemicals have ability to fight against microorganisms or inhibit the growth of microorganisms. This approach will be an advanced step in the discovery of some herbal drugs. These plant extracts which were proven to be potentially effective can be used as a natural alternative to the chemical preservatives frequently used in food preparations. It could be an ideal way to avoid health hazards that may occur due to chemical antimicrobial agents.

Introduction

Microbes are present everywhere in the biosphere and they have both, beneficial or harmful effects with regard to human measure or surveillance. The major harmful effect is food poisoning due to bacterial contamination which causes illness and death in developing countries (Doughari, 2012). Particularly members of Gram negative bacteria such as

Salmonella typhi, *Escherichia coli* and *Pseudomonas aeruginosa*; and Gram positive bacteria such as *Bacillus cereus, Bacillus subtilis and Staphylococcus aureus*; have been identified as causal agents of food spoilage or food borne diseases (Mostafa *et al.,* 2018; Ibrahim and Fagbohun 2013;Lucera *et al.,* 2012).

Antibiotics and chemical preservatives are boon for the control of food poisoning diseases since they are one of the most important weapons to fight against bacterial infections. Their benefits are numerous but over last few years, utilization of chemical preservatives and antibiotics handled to various consequences such as microbial resistance, accumulation of chemical residues in feed and food chain and undesirable side effects (such as nausea, depression of bone marrow, thrombocytopenic purpura and agranulocytosis) on human health (Fair and Tor, 2014; Bialonska *et al.,* 2010). Hence, new efforts and effective techniques are required in order to fight against these problems.

Plants are a very good source of medicinal compounds that play a vital role in human health as they represent a rich source of phytochemical constituent (Mostafa *et al.,* 2018). These phytochemical includes the range of secondary metabolites such as alkaloids, terpenoids, tannins, flavonoids and glycosides, etc., which have a different antimicrobial properties (Doughari, 2012). Various extract from the parts of the plant like root, stem, leaf, fruit and flower have been experimentally shown to have anti-microbial activity and also regarded as nutritionally safe and easily degradable. Hence, the extract of various species of edible and medicinal plants, can be used as the substitute source of synthetic antimicrobials for preservation in food and beverages, and it also offersa great significance in the therapeutic treatments (Hintz *et al.,* 2015; Mbakwem-Aniebo *et al.,* 2017). However, this phytochemical based approach is not much developed compared to the modern system of medicine, due to either lack of or insufficient scientific studies in this area.

Several researchers have demonstrated antimicrobial activity against pathogenic bacteria from the extracts of various plants such as guava, garlic, ginger, cumin, clove, pomegranate etc. (Mostafa *et al.,* 2018; Nuamsetti *et al.,* 2012; Maharjan *et al.,* 2011). In the same line of work, *Moringa oleifera* (drumstick tree),*Vitexnegundo* (five-leaved chaste tree) and *Rosa indica*(rose) were used in the present study.

M. oleifera has been used as traditional medicine for the treatment of several skin diseases; as stimulant in paralytic afflictions, epilepsy and hysteria. The root of this plant is reported to exhibit anti-inflammatory action while its other parts such as leaves, stem and seeds also had demonstrated various therapeutic properties (Kalpana *et al.,* 2013; Dalukdeniya *et al.,* 2016).

V. negundo is a medicinally important plant reported for beneficial properties like anti-inflammatory, anti-asthmatic, antibacterial and antifungal (Rose and Catherine, 2011; Srinivas *et al.,* 2010;Gautam and Kumar, 2012). *Rosa indica* is generally most popular for its beauty, fragrance and antimicrobial as well as antioxidant properties (Halawani *et al.,* 2014; Kumar *et al.,* 2012).

Thus, for extending the understanding about phytochemical properties of *M. oleifera*, *V. negundo* and *R. indica*, the present study was aimed at two main objectives.

First, to assess the antibacterial activity of plant extract (methanol, ethanol and acetone) against gram positive and gram negative bacteria by disc diffusion method and second, to evaluate phytochemical nature of plant extract.

Materials and Methods

Plant materials and bacterial strain

Leaves of *M. oleifera* and *V. negundo*; and petals of *R. indica* were used as plant material for preparation of extract. The antibacterial activity of each plant extract was assessed using three bacterial strains causing food poisoning diseases, two strains of Gram positive (*Staphylococcus aureus* and *Bacillus cereus*) and one strains of Gram negative (*Salmonella typhi*) bacteria.

Preparation of plant extracts

The plant materials, from *M. oleifera*, *V. negundo* and *R. indica* were collected, disinfected, water washed, and dried under a shade. The dried plant material of each plant species was grind using mortar and pestle to obtain fine powder and then was passed through 1.00 mm sieve. 20 gram fine powder of each plant was soaked in 100 ml of different solvents such as methanol and acetone separately for 48 hours followed by loading in soxhlet apparatus and subject to continuous extraction (4-5 hours) with respective solvents to obtain crude extracts. There after the solvent (acetone or methanol) was removed under reduced pressure using rotatory vacuum evaporator (Wang and Weller 2006; Maharjan *et al.,* 2011). The concentrated residues were dissolved in dimethyl sulphoxide (DMSO; 10%w/v) and stored at 4ºC until use (Kalpana *et al.,* 2013). The extract yields were weighted and stored in small bottles in refrigerator at 4ºC. Yield percentages were calculated using the following formula:

Extract yield% = R/S x 100

Where R = weight of extracted plants residues and S = weight of raw plant sample.

Evaluation of antimicrobial activities of plant extracts

Antimicrobial activity of plant extracts was analysed by disc diffusion method as described by Bauer *et al.,* (1966) and Cakir *et al.,* (2004) with minor modification. About 20ml nutrient (base) agar was plated in petri dishes and allowed to solidify for 30 minutes. The test microorganisms such as *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhi* were seeded (0.2 ml: 10^7-10^8cells/ml) into sterile molten nutrient soft agar medium which was overlaid on the nutrient agar base. The filter discs (5 mm diameter) were soaked with extracts (100mg/ml) and then placed on the surface of the seeded agar plates. A filter disc saturated with 10µl of DMSO (Dimethyl sulphoxide) was used as negative control. These plates were incubated at 37˚C for 24-48 hours to allow maximum growth of the microorganism. After incubation, the plates were observed for clear, distinct zone of inhibition surrounding the disc. The diameter (mm) of zone of inhibition produced by the extract was measured and compared with the standard. All assays performed in triplicates to consider mean values as a standard one.

Phytochemical analysis of plant extract:

Test for Tannins

About 1 ml extract (Conc. 10%w/v) was mixed in 3ml water and heated on boiling water for 5 minutes and then filtered. Further, 1 ml of 0.1% ferric chloride was addedto3ml filtrate and observed for the appearance of dark green color or blue- black color. The appearance of this color indicates the presence of tannins (Edeoga *et al.,* 2005).

Test for flavonoids

The different plant extracts (0.5 ml) was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted

residue was dissolved in 20ml of ethanol and filtered. 3 ml of the filtrate was mixed with 4 ml of 1 % potassium hydroxide in a test tube and the color change was observed. A dark yellow color indicates the presence of flavonoids (Hadi and Bremner 2001).

Test for alkaloids

Wagner's test and Mayer's test were performed to study the presence of alkaloids. 0.5 ml of extract was mixed and with 8 ml of 1 % HCl followed by heating in boiling water bath. After cooling, the mixture was filtered. Thereafter 2 ml of the filtrate treated with Mayer's and Wagner's reagents, and observed for the formation of white-yellowish and reddish- brown precipitate respectively (Evans 1997; Wagner 1984).

Results and Discussion

Now a day, immense and indiscriminate utilization of antibiotics for the treatment of infectious diseases is leading to the emergence of drug resistance among etiological agents similarly chemical preservatives are also employed to control food pathogens, but it is influenced by several factors such as high cost and severe side effects. Hence, it is an alarming situation and deserves major attention to develop other promising alternatives (Sharma, 2015; Silva and Lidon, 2016). Plants are the readily available resource of valuable natural therapeutic remedies and have a long history of healing effects and more over a promising alternative to chemical preservatives and medicine. Approximately 3000 plants species in India are known to have medicinal properties (Prakasha *et al.,* 2010). These medicinal plants may prove to be rich source of compounds with possible antimicrobial properties but more antimicrobial and phytochemical investigation is necessary.

In the present study antibacterial activity and phytochemical properties of *M. oleifera*, *V. negundo* and *R. indica*, were investigated. Plant materials were dried and extracted using methanol and acetone to extract valuable compounds.

Plants extraction yield

The ethnobotanical data of the employed plants and their extract percentage yield are illustrated in Table 1. Plant materials with methanol yielded plant extract residues ranged from 24.35 to 9.83% while in case of acetone it ranged from 12.42 to 8.62%.The higher extract yield in methanol may be due to the higher solubility of proteins, carbohydrates and other compounds in methanol compared to acetone (Do *et al.,* 2014). The highest yield of plant extract was obtained from *M. oleifera* followed by *V. negundo* while *R. indica*gives the lowest extract yield.

Phytochemical analysis of plant extract

Phytochemical analysis of *M. oleifera*, *V. negundo* and *R. indica* revealed the presence of phenols, alkaloids, tannins, flavonoids and saponins (Table 1). Whereas xantho proteins were not detected in methanol and acetone extract of all these plants. Similarly, the presence of these phytochemicals has reported by many authors in plant extract of the same plants with various extraction solvents such as chloroform, ethanol, ethyl ether etc. (Bukar *et al.,* 2010, Rose and Cathrine 2011, Gautam and kumar 2012 and Halawani *et al.,* 2014). It is well documented that phytochemical such as phenols, alkaloids, tannins, flavonoids and saponins can act as an antimicrobial compound and play an important role to fight against microbes (Do *et al.,* 2014). Hence these all plant extracts were further used for the analysis of antimicrobial activity against different bacteria by disc diffusion method.

Table.1 The ethnobotanical data of employed plant species and their extract yield (%)

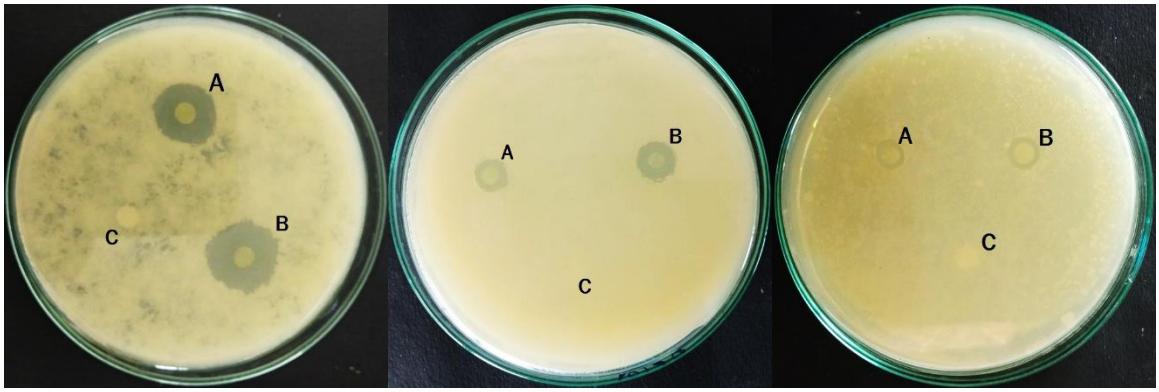
Plant spices	Family	Common Name	Plant part used	Extraction yield (%)	
				Methanol	Acetone
Moringa oleifera	Moringaceae	Drumstick	Leaf	24.35	12.42
Vitex negundo	Verbenaceae	Nirgundi	Leaf	16.67	10.90
Rosa indica	Rosaceae	Rose	Patals	9.83	8.62

Table.2 Phytochemical constituent analysis of methanol and acetone extracts of *R. indica*, *V. negundo* and *M.oleifera*. Where (+) = Present and (-) = absent; High amount = (+++); Relatively high = (++), Trace amount = (+)

Phyto-constituent	Solvent system	Plant extract		
		Moringa oleifera	*Vitex negundo*	*Rosa indica*
Phenolic compounds	Methanol	++	+++	+++
	Acetone	+	++	++
Alkaloids	Methanol	++	++	+++
	Acetone	+	+	++
Tannins	Methanol	++	+++	++
	Acetone	+	++	+
Flavonoids	Methanol	++	+++	++
	Acetone	+	++	+
Xantho proteins	Methanol	-	-	-
	Acetone	-	-	-
Saponins	Methanol	+	++	-
	Acetone	+	+	-

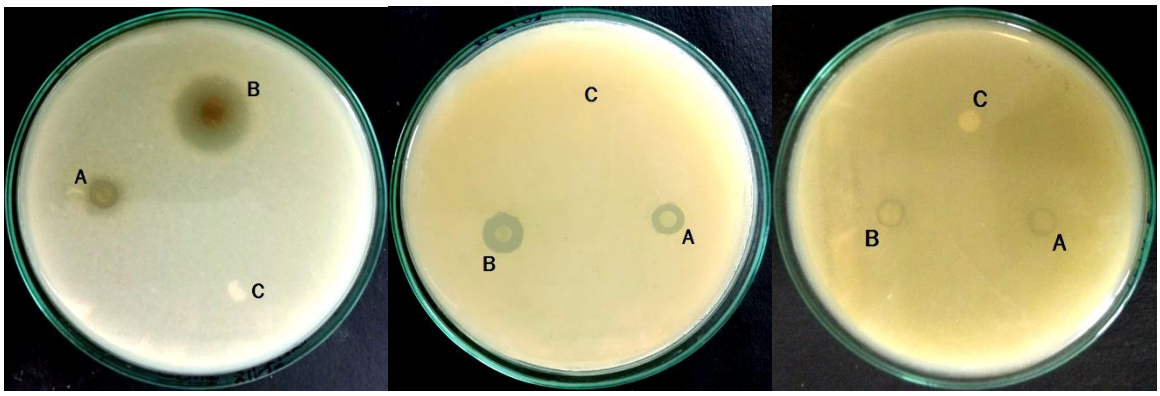
Table.3 Determination of antimicrobial activity of *V. Negundo*, *R. indica* and *M. oleifera* against bacterial pathogens by disc diffusion method. Values represented as mean ± SE (standard error)

Plant sample	Microorganism	Zone of inhibition (mm)	
		Methanol	Acetone
M. oleifera	*B. cereus*	17.83±0.44	14.33±0.88
	S. aureus	13.17±0.60	10.50±0.29
	S. typhi	9.67±0.66	6.83±0.17
V. negundo	*B. cereus*	15.00±0.76	9.00±0.76
	S. aureus	10.33±0.33	7.00±0.29
	S. typhi	6.17±0.44	5.80±0.16
R. indica	*B. cereus*	16.00±1.00	14.00±0.58
	S. aureus	9.17±0.17	7.33±0.37
	S. typhi	7.00±0.29	5.83±0.16



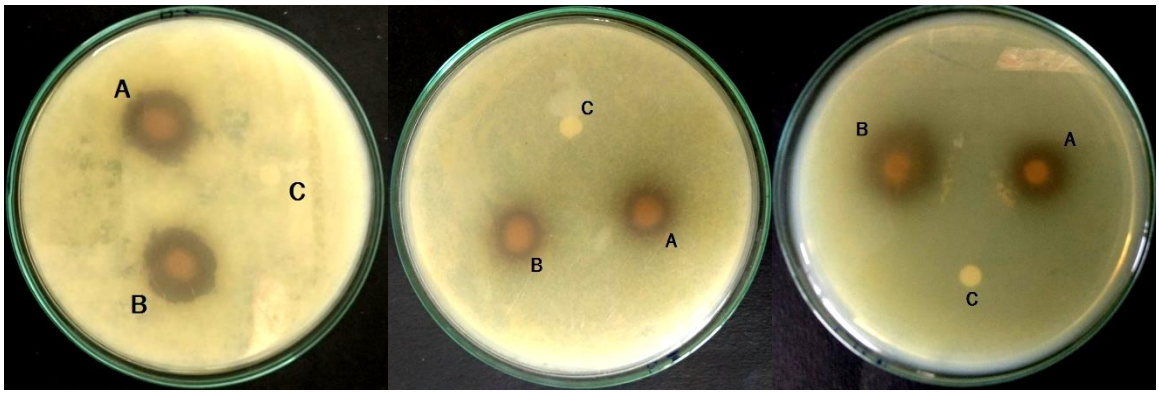
Bacillus cereus *Staphylococcus aureus* *Salmonella typhi*

Fig.1a - Effect of *Moringa oleifera* extracts on different bacteria.
A) Acetone extract B) Methanol extract C) Control



Bacillus cereus *Staphylococcus aureus* *Salmonella typhi*

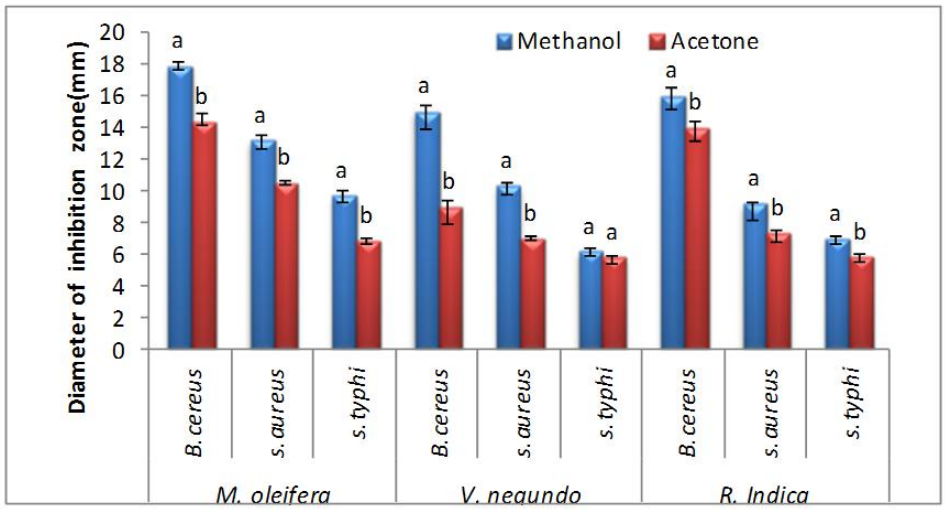
Fig.1b - Effect of *Vitex negundo* extracts on different bacteria.
A) Acetone extract B) Methanol extract C) Control



Bacillus cereus *Staphylococcus aureus* *Salmonella typhi*

Fig.1c - Effect of *Rosa indica* extracts on different bacteria.
A) Acetone extract B) Methanol extract C) Control

Fig.2 Comparison of methanolic and acetonic extract of *M. oleifera and V. negundo, R. indica* against bacterial pathogens. Graph represents the mean ± SE (n = 3) followed by similar lower case letter are significantly not different according to Tukey's multiple range at P≤0.05



The extracts from *M. oleifera, R. indica and V. negundo* showed varying degrees of antimicrobial activity against the different test organisms (Table 3) while there was no inhibition of growth with the control (DMSO) as it used as negative control (Fig. 1). Methanolic and acetonic extract from *M. oleifera* showed higher zone of inhibition against different test organisms in a rage of 17.83 – 9.67 mm and 14.33 – 6.83 mm respectively (Table 3; Fig. 2). Similarly *R. indica* and *V. negundo* also exhibited antimicrobial activity against different test organisms. Overall, it was observed that methanolic extract exhibited significant higher ($p \leq 0.05$) antibacterial activity against all test organisms as compared to acetonic extract (except in case of *V. negundo against S. typhi*). All three plants extracts (methanolic and acetonic) showed comparative elevated antimicrobial activity against *B. cereus* followed by *S. aureus and S. typhi*. Methanolic extract from *M. oleifera* showed 24%, 25% and 41% higher activity against *B. cereus, S. aureus and S. typhi* compared to acetonic extract respectively (Fig. 2).

Similarly, elevated antimicrobial activity in methanolic extract was reported in *M.oleifera* (Kalpana *et al.,* 2013), *Phyllanthus niruri* (Shanmugam *et al.,* 2014), *Simmondsia chinensis*, *Jatropha curcas*, *Zingiber officinale* and *Syzygium aromaticum* (Ibrahim and Abu-Salem 2014) compared to aqueous extract. In addition, extract yield in methanol solvent was significantly higher; therefore it may enhance the solubility of active components of *M. oleifera, R. indica* and *V. negundo* which resulted in higher antimicrobial activity compared to acetone extract. Results of antimicrobial activity of the three plant extracts suggested that *S. typhi* was more resistant strain to plant extracts as compared to *B. cereus* and *S. aureus.* As *S. typhi* contains an outer lipopolysaccharide layer, it may hinder the access of most components to the peptidoglycan layer of cell wall (Srinivas *et al.,* 2010). This could be the possible reason for lower activity of different plant extracts against *S. typhi*.

In conclusion, this study reports the presence of various phytochemical constituents such as alkaloids, tannins and phenolic compound in different solvent extracts (methanol, acetone) of *V. negundo, R. indica* and *M. oleifera*. Among both extraction solvents methanol gives higher extraction yield for all three plants. Methanolic and acetonic extract from these plants offered a significant antimicrobial activity to test organism show ever methanolic extract from these plants showed comparative higher antimicrobial activity. In addition to this study further efforts including quantification, purification, detection of toxicity and side effects of antimicrobial compounds, may be required to strengthen potential this antimicrobial plant extract and favourable outcomes.

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