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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF *STRYCHNOS NUX-VOMICA* (L)

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ABSTRACT

The present study was carried out that preliminary phytochemical analysis and antibacterial activity was carried out in *Strychnos nux-vomica* against four selected pathogenic bacteria by disc diffusion method. The preliminary phytocompound such as carbohydrate, tannin and phenolic compounds, flavonoids and phytosterol shows the presence of chloroform, ethyl acetate and ethanol leaf extract of *Strychnos nux-vomica*. Where as in phytosterol compound is absent in chloroform leaf extract. In antibacterial activity, Ethanol leaf extract exhibit higher activity in *Salmonella typhi* when compared to Chloroform and Ethyl acetate leaf extract against *Escherichia coli.* In this Endeavour, traditional herbal medicines must perforce grant the benefits of modern science and technology to serve further global needs. The drugs derived from herbs may have the possibility of their use in medicine because of their good antibacterial activity.

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INTRODUCTION

India is a varietal emporium of medicinal plants is and one of the richest countries in the world in regard to genetic resources of medicinal plants. They played an essential role in the development of human culture. Plants are natures, "Chemical factories" providing the richest source of organic chemicals on earth. Studying medicinal plants have been used for years to treat disease all over the world. In recent years, many possible sources of natural antibiotics have been in used for several infectious diseases, mostly bacteria and fungi. Many works have been done which aim at knowing the different antimicrobial and phytochemical constituents of medical plants and using them for the treatment of microbial infections as possible alternatives to chemically synthetic drugs to which many infections microorganism have become resistant. Nature has been a source of medicinal agent for thousands of years and impressive number of modern drugs has been isolated from natural sources. (Owolabi *et al.,* 2007). Traditional medicines are readily available in rural areas at relatively cheaper than modern medicine. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Varaprasad Bobbarala *et al.*, 2009). Medicinal plants are the richest sources of drugs of traditional systems of medicine, modern medicines, nutraceutical, food supplements, folk medicine, pharmaceuticals intermediates and chemical entities for synthetic drugs (Hammer *et al.*, 1999). Medicinal plants are a source of great economic value all over the world. The WHO in 1997 suggested that effective locally available plants be used as substitutes for drugs. The aim of the work is to analyses the antibacterial activity and preliminary phytochemical analysis of leaf extracts of *Strychnos nux – vomica* (L) using various solvent such as chloroform, ethyl acetate and ethanol against four pathogenic bacteria.

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MATERIALS AND METHODS

Plant materials

The plant materials were collected from the Rasipuram Tk, Namakkal District, Tamil nadu.

Medicinal Properties of Plant

The fresh leaves are applied as a poultice on wounds and ulcers. It is used in frurities and as a local anodyne in inflammations of the external ear. And as prescribed for digestive problems, sensitivity to cold, and irritability. It improves the pulse and raises blood pressure and is of great value as a tonic to the circulatory system in cardiac failure.

Preparation of Plant Extract

The plant materials were collected and it was shade dried for one week after drying the plant materials were used for experiments. 50g of dried plant material was powdered with the help of mixer grinder and it was soaked with the respective solvents such as Chloroform, Ethyl acetate and Ethanol for 48 hrs at 30^{O} C. After 48 hrs the extracts were filtered with the help of filter paper and it is stored in clean container. It was used for further analysis.

Disc diffusion method

The disc diffusion method provides a simple and reliable test in routine clinical microbiology. In order to find out the effect of a particular substance on a specific bacteria. This method consists of impregnating small circular disc of standard filter paper with given amount of a chosen concentration of substance. The discs are placed on plates of culture medium previously spread with bacterial inoculums to be tested. After incubation the degree of sensitivity is determined by measuring the inhibition zone produced by the diffusion of the antibiotic substances from the discs into the surrounding medium (Maruzzella and Henry, 1958).

Preparation of discs

Discs usually consist of absorbent paper impregnated with the compound plant extract. 6mm (diameter) discs were prepared from Whatman No. 1 filter paper, the discs were sterilized by autoclave at 121^{O} C. There dry discs were used for the assay.

Procedure

Circular discs of 6mm (diameter) were prepared from Whatman No. 1 filter paper and sterilized in an autoclave. These discs were impregnated with extracts in the respective solvents for overnight and placed on nutrient agar plates with tested bacteria. The plates were incubated at 37^{O} C for 24 hrs. 1mm and the values are recorded and tabulated the incubation period zone inhibition was measured. Here various extracts are used to bacteria, like Ethyl acetate, Ethyl and chloroform extracts for leaves. Separate discs were prepared by impregnating with only solvent as control. The values of negative control are deducted from the tested value for analysis. The tests were repeated 3 times to ensure reliability of the result. Premilinary Phytochemical screening was carried out using the methods adopted by (Wagner *et al*., 1984; Harbone, 1973).

Statistical analysis

Data were expressed as Mean ± Standard deviation. The data obtained were subjected to ANOVA test to determine whether there was significant difference between extract and also between the lengths of incubation.

RESULTS AND DISCUSSION

Preliminary Phytochemical Studies:

The preliminary phytochemical analysis (Wagner *et al*., 1984) and Harborne, (1973) were carried out in Chloroform, Ethyl acetate and Ethanol extract of *Strychnos nux vomica*. It revealed that the presence of carbohydrate, Tannins, phenolic compounds, flavonoids and phytosterol. In phytosterol, it reveals the presence in ethyl acetate and ethanol and absent in chloroform. These leaf extracts showed the absence of Glycosides, saponins, protein, amino acid, Alkaloids, Gum and mucilage. There compounds have been reported to inhibit bacteria growth and capable for protecting against bacterial infections. (Table 1) Previously the observation was done by Dinesh *et al*. (2012) shows the presence of alkaloid, carbohydeate, tannin, steroid, triterpenoid, glycoside.

Table 1. Phyto chemical studies of *strychnos nux vomica* leaf various extracts

S.No	Experiment	Chroloform Extract	Ethyl Acetate Extract	Ethanol Extract
1	Carbohydrates			
	Molisch's Test	+	+	+
	Fehling's Test	-	-	-
2	Saponins Test	-	-	-
3	Proteins and Aminoacids			
	Millon's Test	-	-	-
	Biuret Test	-	-	-
	Ninhydrine Test	-	-	-
4	Tannin and Phenolic Compounds	+	+	+
5	Phytosterol Test	-	+	+
6	Alkaloids			
	Mayer's Test	-	-	-
	Dragendroff's Test	-	-	-
7	Gums and Mucilage	-	-	-
8	Flavonoids	+	+	+

Antibacterial Activity

Infection diseases are the major cause of morbidity and mortality worldwide. The number of multidrug resistant microbial strains and the appearance of strains which reduced susceptibility to antibiotics are continuously increasing. Such increase has been attributed to indiscriminate use of broad spectrum antibiotics, immunosuppressive agents, intravenous catheters organ transplantation and ongoing epidermis of human immunodeficiency virus (HIV) infection. This situation provides the impetus to the search for new antimicrobial substances from various sources like medicinal plants.

The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contribution to human heath and well being. The use of plant extract with known antimicrobial properties can be of great significance for therapeutic treatment. (Selvamohan, 2012). The antibacterial activity of Chloroform, Ethyl acetate and Ethanol extracts of *Strychnos-nux vomica* was investigated using disc diffusion method against selected pathogens such as *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pnemoniae.* These four different pathogens have also tested with commercially available antibiotic (tetracycline) and results were indicated in Table 2, 3 & 4.

Effect of Chloroform Leaf Extract

The antibacterial activity of Chloroform leaf extract of *Strychnos-nux vomica* against four different pathogenic

bacteria using disc diffusion method have showed maximum zone of inhibition against *Escherichia coli* followed by *Klebsiella Pneumoniae*. The moderate in effect is observed in *Salmonella typhi* and minimum activity found in *Pseudomonas aeruginosa*. (Table 2, Fig. 1 and Plate1).

Table 2. Effect of Chloroform leaf extract of *Strychnos nux – vomica* by disc diffusion method

Microorganisms	Diameter of Inhibition Zone in mm (mean)*	
	Chloroform Extract	Standard antibiotic (#)
Escherichia coli	0.5 ± 0.35	8.1 ± 0.02
Pseudomonas aeruginosa	0.057 ± 0.04	7.0 ± 0
Salmonella typhi	0.15 ± 0.10	2.1 ± 0.01
Klebsiella Pneumoniae	0.43 ± 0.30	7.0 ± 0

* Mean for triplicate
± Standard deviation
Tetracycline

Table 3. Effect of ethyl acetate leaf extract of *Strychnos nux – vomica* by disc diffusion method

Microorganisms	Diameter of Inhibition Zone in mm (mean)*	
Escherichia coli	Ethyl Acetate Extract	Standard antibiotic (#)
Pseudomonas aeruginosa	0.28 ± 0.20	7.0 ± 0
Salmonella typhi	0.46 ± 0.32	2.1 ± 0.01
Klebsiella Pneumoniae	0.57 ± 0.40	7.0 ± 0

* Mean for triplicate
± Standard deviation
Tetracycline

Table 4. Effect of ethanol leaf extract of *Strychnos nux – vomica* by disc diffusion method

Microorganisms	Diameter of Inhibition Zone in mm (mean)*	
Escherichia coli	Ethyl Acetate Extract	Standard antibiotic (#)
Pseudomonas aeruginosa	0.57 ± 0.40	7.0 ± 0
Salmonella typhi	1.15 ± 0.81	2.1 ± 0.01
Klebsiella Pneumoniae	0.28 ± 0.20	7.0 ± 0

* Mean for triplicate
± Standard deviation
Tetracycline

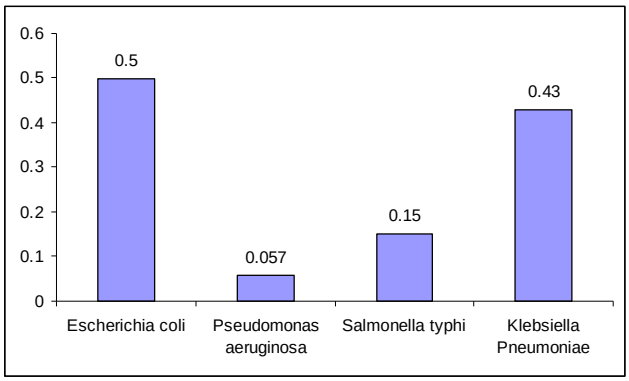


Figure 1. Effect of Chloroform leaf extract of *Strychnos nux – vomica* by disc diffusion method

Effect of Ethyl Acetate Leaf Extract

The leaf Ethyl acetate extract of *Strychnos-nux vomica* rate of inhibition in Escherichia coli against rest of the pathogenic organisms. The better inhibition effect is observed in *Klebsiella Pneumoniae.* The moderate inhibitory effect is observed in *Salmonella typhi.* The minimum inhibitory effect is observed in *Pseudomonas aeruginosa* (Table 3, Fig. 2 and Plate 2).

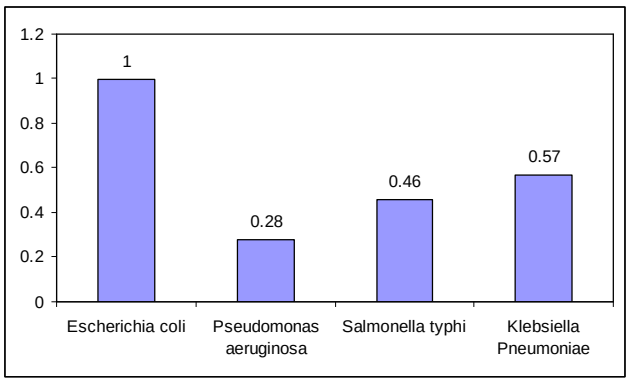


Figure 2. Effect of ethyl acetate leaf extract of *Strychnos nux – vomica* by disc diffusion method

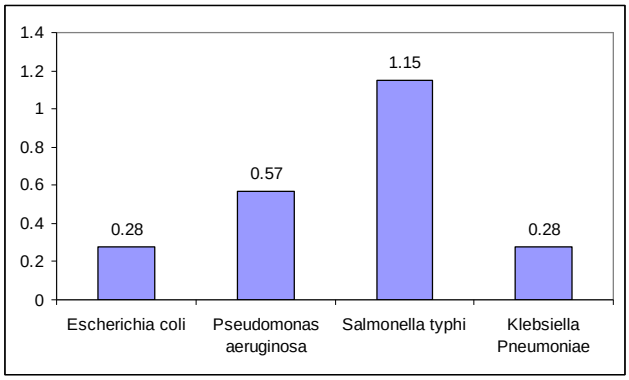


Figure 3. Effect of ethanol leaf extract of *Strychnos nux – vomica* by disc diffusion method

Effect of Ethanol Extract

The antibacterial activity of Ethanol extract of leaf against four pathogenic organisms showed the maximum inhibitory effect in *Salmonella typhi*. The *Pseudomonas aeruginosa* lies in the moderate zone of inhibitory effect. The minimum inhibitory activity found in *Escherichia coli* and *Klebsiella pneumonia.* (Table 4, Fig. 3 and Plate 3). The antibacterial potency may be due to the presence of many potent compounds such as carbohydrate,tannin and phenolic compound and flavonaids. The results showed that Ethanol extract of leaf possess antibacterial activity against *salmonella typhi* The result in chloroform and Ethyl acetate extract of leaf posses antibacterial activity against *Escherichia coli* is less when compared to ethanol extract against *Salmonella typhi.* In this present study, preliminary screening for antibacterial activity showed, that Ethonal leaf extract of *Strychnos nux-vomica* exhibited maximum inhibitory zone against *Salmonella typh*i while the chloroform and ethyl acteate extracts showed least inhibitory activity.

Similar observation as done by Gnanavel *et al*. (2012) shows the maximum zone of inhibition in n-butanol extract against

PLATE 1. Antibacterial activity on chloroform leaf extarct of *Strychnos nux Vomica* l. by disc diffusion method

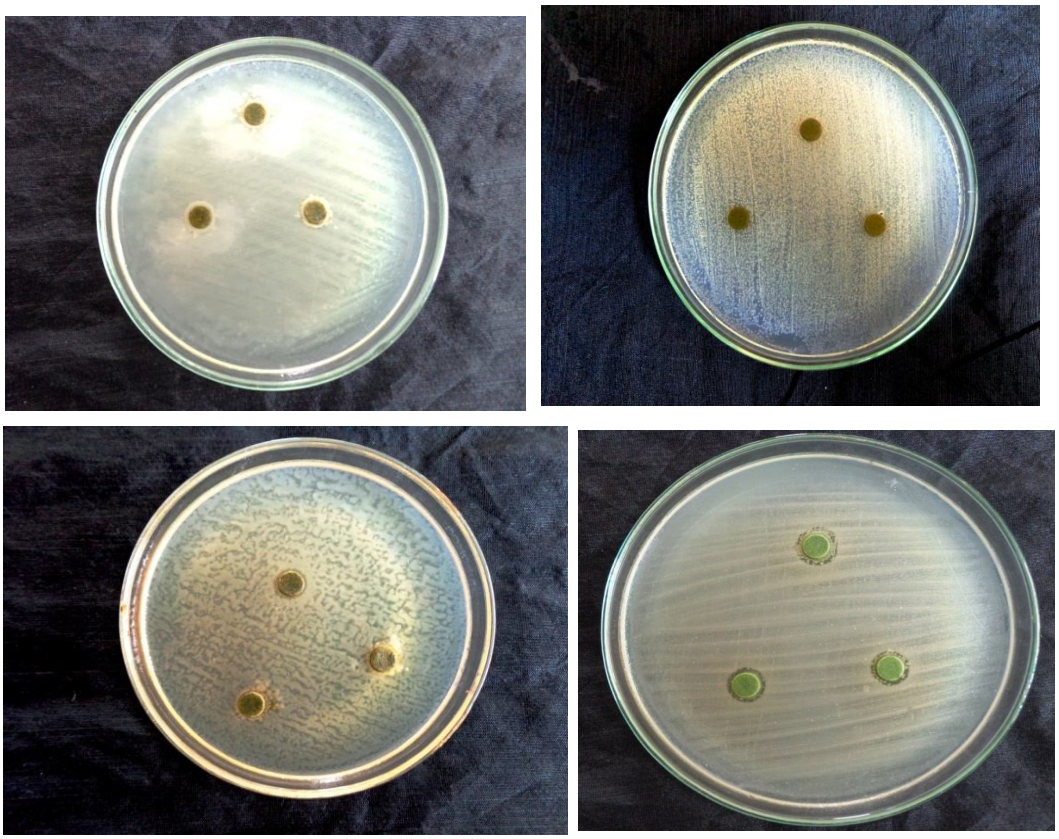


Plate 2. Antibacterial activity on ethyl acetate leaf extarct of *strychnos nux vomica* l. by disc diffusion method

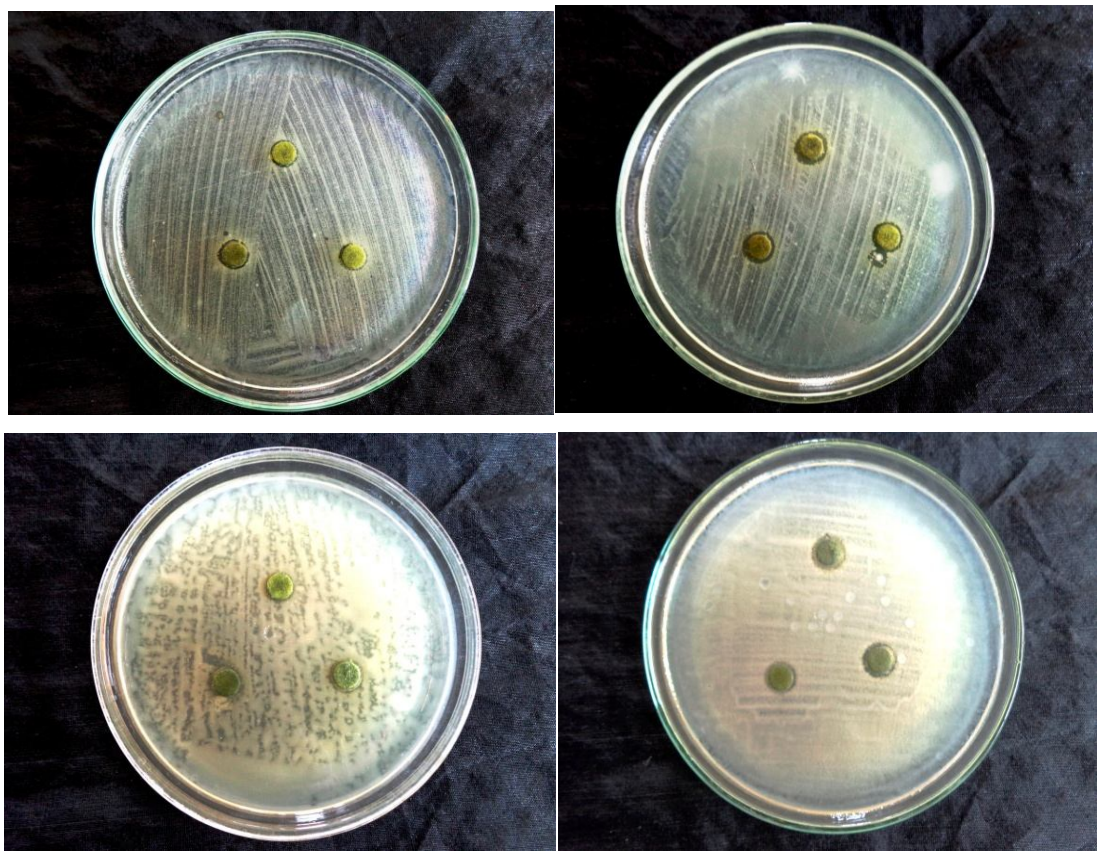
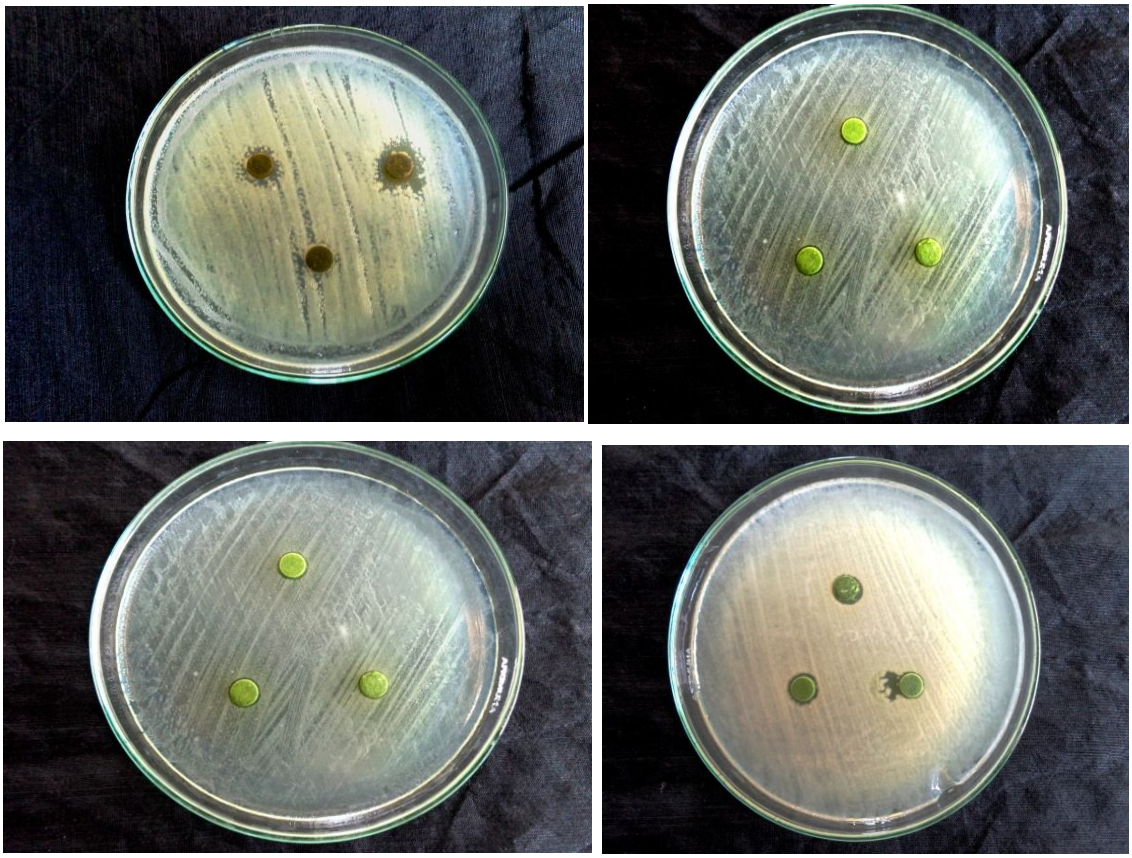


Plate 3. Antibacterial activity on ethanol leaf extarct of *strychnos nux vomica* l. by disc diffusion method



Staphylococcus aureus by using the leaf of *Strychnos nux-vomica*. In *Rhodedendron setesum* shows maximum zone in Escherichia coli by using Ethanolic extracts (Himal Paudel Chhetri *et al.,* 2008). And also reported in Muthukumaran *et al.* (2011) by using methanol extracts of various plants**.**

Summary and Conclusion

The present study was concluded that preliminary phytochemical analysis and antibacterial activity was carried out in *Strychnos nux-vomica* against four selected pathogenic bacteria by disc diffusion method. The preliminary phytocompound such as carbohydrate, tannin and phenolic compounds, flavonoids and phytosterol shows the presence of chloroform, ethyl acetate and ethanol leaf extract of Strychnos *nux-vomica*. Where as in phytosterol phytocompound is absent in chloroform leaf extract.

In antibacterial activity, Ethanol leaf extract exhibit higher activity in *Salmonella typhi* when compared to Chloroform and Ethyl acetate leaf extract against *Escherichia coli*. Minimum zone of inhibition was showed in *Pseudomonas aeruginosa* in chloroform leaf extract when compared to Ethyl acetate and Ethanol. In this Endeavour, traditional herbal medicines must perforce grant the benefits of modern science and technology to serve further global needs.

The drugs derived from herbs may have the possibility of their use in medicine because of their good antibacterial activity. In future, this study will be helpful for the quantitative determination of phyto constituents.

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