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Evaluation of antibacterial potential of *Ficus species*

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Abstract

Aim: The present study was designed to evaluate the *in vitro* antibacterial activities of methanol extract of leaves of four species of *Ficus i.e. Ficus religiosa, Ficus benghalensis, Ficus elastica, Ficus benjamina* and *Ficus infectoria* against four Gram negative bacterial strains *i.e. Escherichia coli* MTCC 40, *Pseudomonas aeruginosa* MTCC 424 and *Salmonella typhimurium* TA98 and TA100.

Methods: Leaf methanol extracts of five *Ficus species* were prepared by using standardized protocols to study the antibacterial effect against different bacterial strains such as *E. coli* MTCC 40, *Pseudomonas aeruginosa* MTCC 424 and *Salmonella typhimurium* TA98 and TA100 by using disc diffusion and luria broth dilution method.

Results: It was observed that among all the plants used in the present study, the methanol extract of *Ficus benghalensis* exhibited the maximum activity against *Salmonella typhimurium* TA98 and *Pseudomonas aeruginosa* MTCC 424. *Ficus religiosa* showed maximum antibacterial activity against *Pseudomonas aeruginosa* MTCC 424 and *Ficus elastica* showed antibacterial activity against *Escherichia coli* MTCC 40 and *Salmonella typhimurium* TA100. Luria broth dilution assay was also used to determine the minimum inhibitory concentration of plant extracts. It was found that methanol extract of *Ficus religiosa* showed maximum antibacterial effect against *Salmonella typhimurium* TA100 and *Escherichia coli* MTCC 40.

Conclusion: The results showed antibacterial activities of *Ficus religiosa* as well as that of *Ficus benghalensis.* It provided the scientific basis for the development of new pharmaceuticals from the plants as well as confirmed the traditional use of these plants in the treatment of bacterial infections.

Keywords: *Ficus*; Bacterial infections; Luria broth dilution assay; Disc diffusion assay

INTRODUCTION:

Overuse of antibiotics has made bacteria drug resistant especially infection causing gram – ve bacteria [1]. Drug resistant bacteria are the main causes of bacterial infections, especially Gram-negative bacteria [1]. Medicinal plants are the rich source of antibacterial agents. From ancient times, many plants are used to prepare different types of medicines to cure bacterial infections. *Ficus religiosa, Ficus benghalensis, Ficus elastica, Ficus benjamina* and *Ficus infectoria* are important ingredients of many medicinal formulation in Ayurveda. *Ficus* represents more than 800 species and are used to treat various bacterial infections and diseases that are the leading cause of morbidity and mortality worldwide. According to WHO report, approximately 80% of the world population rely on plants or plant derived products for the treatment of diseases [2]. Keeping in mind the various medicinal uses of genus *Ficus* the present study was designed to investigate the *in vitro* antibacterial activities of methanol extract of leaves of *Ficus religiosa, Ficus benghalensis, Ficus elastica, Ficus benjamina* and *Ficus infectoria* against four Gram negative bacterial strains *i.e. Escherichia coli* MTCC 40, *Pseudomonas aeruginosa* MTCC 424 and *Salmonella typhimurium* TA98 and TA100 using standard methods.

MATERIALS AND METHODS:

Plant material and extraction preparation

The leaves of different species of *Ficus* were obtained from the trees growing in the Botanical Garden of Guru Nanak Dev University, Amritsar. The Plants were identified at herbarium of Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar where vouchers specimens were deposited. The collected leaves were washed with tap water to clean the dust and impurities; dried at room temperature and then ground to powder form. The obtained powder (500 g) was extracted with 80% methanol for 24 hours with intermittent shaking and the suspended material was filtered using Whatman no. 1 filter paper. The filtrate was collected. The residue obtained was again suspended in the fresh solvent and the procedure was repeated thrice. The filtrate obtained after each filtration was combined and then concentrated under reduced pressure using vacuum rotary evaporator (45ºC) to obtain gummy residue which was lypholized using lypholizer to form dry powder. The extracts were stored at 4ºC until further use.

Procurement of strains

The bacterial strains used for the present study were obtained from Microbial Type Culture Collection (MTCC) and Institute of Microbial Technology (IMTECH). All bacterial strains were maintained as stock strains in cryo vials and kept at -80°C until further use.

Antibacterial assays

Dilution of plant extracts

Plant extracts were (80% methanol) taken and dissolved in dimethyl sulphoxide (DMSO) in order to make different concentrations *i.e.* 40 mg/ml, 30 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml and 2 mg/ml.

Preparation of inoculum

Bacterial strains were streaked over Luria broth (LB) agar plates and incubated at 37°C for 24 hours. Single colonies were selected and inoculated in Luria broth at 37°C overnight. The density of inoculum required for the test was adjusted to Mc Farland Standard 0.5 [3].

Preparation of discs

The Whatman No.1 filter paper was used for the preparation of discs and then subjected to different concentrations of extracts and dried at room temperature overnight under aseptic conditions.

Determination of antibacterial activities

This was done using (i) Disc diffusion method (ii) Luria broth dilution (LB) method.

Disc diffusion method

Disc diffusion assay was done to determine the antibacterial activity of plant extracts. The method given by Kirby and Bauer was used [4]. The test tube containing 0.1ml inoculum with 2 ml top agar was spread on nutrient agar medium. The discs impregnated with different concentrations of plant extracts were placed on the nutrient agar plate with the help of sterile forcep. Plates were incubated at 37°C overnight. The zone of inhibition was measured using scale. In this assay, Amoxicillin was used as positive control, whereas DMSO was used as negative control.

General calculations

The effectiveness of different extracts can be calculated by following formula:

$$R = \frac{\text{Area defined by zone of inhibition}}{\text{Area defined by paper disc}}$$

$$R = \frac{\Pi(r)^2}{\Pi r^2}$$

R = relative magnitude of inhibition.
(r) = radius of zone of inhibition
r = radius of paper disc
Π = Pi (3.14)

Luria broth dilution method

Minimum inhibitory concentrations (MIC) was determined using Luria broth dilution method. In this, 10 test tubes containing luria broth was taken and different concentrations of plant extracts were added in them. Test tubes were then inoculated with bacterial suspension and incubated at 35°C for 18 - 24 hours. The turbidity of the culture was observed as turbidity value using turbidity meter (Aqualytic Germany). The lowest concentration of extract that inhibited the growth of bacteria was considered as MIC value for each of the bacterial strains.

$$\text{Percentage inhibition}(\%) = \frac{\text{Control mean - Sample mean}}{\text{Control mean}}$$

RESULTS:

The antibacterial activity of methanol extract of *Ficus religiosa* is shown in Table 1. It was observed that the extract exhibited dose dependent increase in effect for all the microorganisms used in the present study. Furthermore, the extract was found to be ineffective at 2 mg/ml concentration. As the concentration of the extract was increased, the zone of inhibition also increased. *Ficus religiosa* was found to be most effective against *Pseudomonas aeruginosa* with (3.0 mm zone of inhibition) at a concentration of 40 mg/ml and 2.5 mm zone of inhibition at concentration of 30 mg/ml. The order of inhibition at concentration 40 mg/ml was observed to be as follows: *Pseudomonas aeruginosa* [3.0 mm] > *Escherichia coli* [2.0 mm] = *Salmonella typhimurium* (TA98) [2.0 mm] = *Salmonella typhimurium* (TA100) [2.0 mm].

In case of *Ficus benghalensis*, it was observed that methanol extract of leaves was most effective against *Salmonella typhimurium* (TA98) with zone of inhibition (3.5 mm) at concentration of 40 mg/ml and showed negligible activity at concentration 2 mg/ml (Table 2). It was further found that in *Pseudomonas aeruginos* , the extract concentration 40 mg/ml was more effective having zone of inhibition 2.5 mm, while no effect was seen against *Escherichia coli*, *Salmonella typhimurium* (TA98) and *Salmonella typhimurium* (TA100). At highest concentration of 40 mg/ml, the order of potency of the extract against different strains was: *Salmonella typhimurium* (TA98) [3.5 mm] > *Pseudomonas aeruginosa* [2.5 mm] > *Escherichia coli* [2.0 mm] > *Salmonella typhimurium* **(**TA100) [1.0 mm] respectively.

Table 3 showed the antibacterial activity of the methanol extract of *Ficus elastica.* It was found to be most effective against *Escherichia coli* with zone of inhibition (3.0 mm) and *Salmonella typhimurium* (TA100) having zone of inhibition (3.0 mm) at a concentration of 40 mg/ml. At concentration 2 mg/ml, all the strains showed more resistance. It was further noticed that in *Pseudomonas aeruginosa* a good inhibitory effect (2.0 mm) was observed at 40 mg/ml, while no inhibitory effect was seen in *Salmonella typhimurium* (TA98) at the concentration of 5 mg/ml and 10 mg/ml. The order of inhibition at concentration of 40 mg/ml was observed to be as follows: *Escherichia coli* [3.0 mm] = *Salmonella typhimurium* (TA100) [3.0 mm] > *Pseudomonas aeruginosa* [2.0 mm] > *Salmonella typhimurium* (TA98) [1.0 mm].

The results of antibacterial activity of *Ficus benjamina* is shown in Table 4. The results indicated that the methanol extract of leaves was found to be most effective against *Salmonella typhimurium* (TA98) at concentration of 40 mg/ml. At highest concentration of 40 mg/ml, the order of potency in the form of inhibition zone of the extract against different strains was: *Salmonella typhimurium* (TA98) [3.0 mm] > *Pseudomonas aeruginosa* [2.0 mm] > *Salmonella typhimurium* (TA100) [1.0 mm] = *Escherichia coli* [1.0 mm] respectively.

The antibacterial activity of the methanol extract of *Ficus infectoria* against all the tested bacterial strains is shown in Table 5. It was found that extract showed good activity (2.0 mm) against all the strains at 40 mg/ml. Even at lowest concentration (2 mg/ml), *Salmonella typhimurium* (TA98) and *Pseudomonas aeruginosa* showed the inhibition zone of (1.0 mm). At concentration of 4 mg/ml extract showed same effect against all the bacterial strains.

The methanol extract of leaves of *Ficus religiosa* inhibited the growth of *Salmonella typhimurium* (TA100) and *Escherichia coli.* It was noticed that the extract showed dose dependent effect to prevent the growth of all the tested bacteria. In case of *Salmonella typhimurium* (TA98) and *Pseudomonas aeruginosa* the inhibition was up to a concentration of 40 mg/ml and afterwards no effect was found on the growth of bacteria (Table 6). The effect of methanol extract of leaves of *Ficus religiosa* in the form of minimum inhibitory concentration (MIC) was observed to be as follows: *Salmonella typhimurium* (TA100) [10 mg/ml] = *Escherichia coli* [10 mg/ml] > *Pseudomonas aeruginosa* [20 mg/ml] > *Salmonella typhimurium* (TA98) [40 mg/ml].

The order of potency of methanol extract of leaves of *Ficus religiosa* in the form of percentage of inhibition against different tested bacterial strains was in the following order: *Escherichia coli* [47%] > *Salmonella typhimurium* (TA98) [40%] > *Salmonella typhimurium* (TA100) [39%] > *Pseudomonas aeruginosa* [38%].

In *Ficus benghalensis* the extract exhibited dose dependent increase in preventing the growth of *Pseudomonas aeruginosa* and *Salmonella typhimurium* (TA98). The *Ficus benghalensis* was found completely ineffective to inhibit the growth of *Salmonella typhimurium* (TA100) and *Escherichia coli*. The effect of methanol extract of leaves of *Ficus benghalensis* in the form of minimum inhibitory concentration was observed to be as follows: *Pseudomonas aeruginosa* [20 mg/ml] = *Salmonella typhimurium* (TA98) [20 mg/ml] > *Salmonella typhimurium* (TA100) [NA] = *Escherichia coli* [NA].

Table 1: Antibacterial activity of leaf extract of *Ficus religiosa* against different bacterial strains in disc diffusion assay

Concentration of extract (mg/ml)	*E. coli* (40)	*Salmonella typhimurium* (TA98)	*Salmonella typhimurium* (TA100)	*Pseudomonas aeruginosa* (424)
	Inhibition zone (mm)			
2	NA	NA	NA	NA
5	NA	NA	1.4±0.05	1.5±0.05
10	NA	NA	1.5±0.15	2.2±0.05
20	NA	1.0±0.00	2.0±0.05	2.3±0.10
30	1.0±0.05	1.0±0.00	2.0±0.01	2.5±0.05
40	2.0±0.10	2.0±0.05	2.0±0.05	3.0±0.01
Negative control (DMSO)	NA	NA	NA	NA
Positive control (Amoxicillin)	3.0±0.10	2.0±0.05	2.0±0.10	3.0±0.05

Table 2: Antibacterial activity of leaf extract of *Ficus benghalensis* by disc diffusion method in the form of inhibition zone (mm) against different bacterial strains.

Concentration of extract (mg/ml)	*E. coli* (40)	*Salmonella typhimurium* (TA98)	*Salmonella typhimurium* (TA100)	*Pseudomonas aeruginosa* (424)
	Inhibition zone (mm)			
2	NA	NA	NA	1.9±0.10
5	NA	1.0±0.05	NA	2.1±0.10
10	NA	1.0±0.10	NA	2.3±0.10
20	1.0±0.01	2.0±0.00	NA	2.3±0.00
30	1.0±0.00	2.5±0.10	1.0±0.10	2.4±0.10
40	2.0±0.00	3.5±0.00	1.0±0.10	2.5±0.05
Negative control (DMSO)	NA	NA	NA	NA
Positive control (Amoxicillin)	3.0±0.01	2.0±0.05	2.0±0.10	3.0±0.60

Table 3: Antibacterial activity of leaf extract of *Ficus elastica* by disc diffusion method in the form of inhibition zone (mm) against different bacterial strains.

Concentration of extract (mg/ml)	*E. coli* (40)	*Salmonella typhimurium* (TA98)	*Salmonella typhimurium* (TA100)	*Pseudomonas aeruginosa* (424)
	Inhibition zone (mm)			
2	NA	NA	NA	NA
5	NA	NA	1.0±0.17	1.0±0.10
10	1.0±0.11	NA	1.0±0.05	1.0±0.05
20	2 0.7±0.10	1.0±0.00	1.0±0.11	2.0±0.10
30	2.8±0.05	1.0±0.05	2.7±0.05	2.0±0.20
40	3.0±0.05	1.0±0.11	3.0±0.05	2.0±0.00
Negative control (DMSO)	NA	NA	NA	NA
Positive control (Amoxicillin)	3.0±0.05	2.0±0.00	2.0±0.10	3.0±0.05

Table 4 : Antibacterial activity of leaf extract of *Ficus benjamina* in the form of inhibition zone (mm) against different bacterial strains.

Concentration of extract (mg/ml)	*E. coli* (40)	*Salmonella typhimurium* (TA98)	*Salmonella typhimurium* (TA100)	*Pseudomonas aeruginosa* (424)
	Inhibition zone (mm)			
2	1.0±0.10	NA	NA	NA
5	1.0±0.00	NA	1.0±0.05	1.0±0.00
10	1.0±0.05	1.0±0.00	1.0±0.00	1.0±0.11
20	1.0±0.00	1.0±0.05	1.0±0.10	1.0±0.17s
30	1.0±0.05	1.0±0.10	1.0±0.10	2.0±0.00
40	1.0±0.00	3.0±0.10	1.0±0.05	2.0±0.05
Negative control (DMSO)	NA	NA	NA	NA
Positive control (Amoxicillin)	3.0±0.10	2.0±0.00	2.0±0.17	3.0±0.11

Table 5 : Antibacterial activity of leaves of *Ficus infectoria* in the form of inhibition zone (mm) against the different bacterial strains.

Concentration of extract (mg/ml)	*E. coli* (40)	*Salmonella typhimurium* (TA98)	*Salmonella typhimurium* (TA100)	*Pseudomonas aeruginosa* (424)
	Inhibition zone (mm)			
2	NA	1.0±0.00	NA	1.0±0.00
5	1.0±0.05	1.0±0.00	NA	1.0±0.05
10	1.0±0.00	1.0±0.05	NA	1.0±0.30
20	2.0±0.10	1.0±0.30	NA	1.0±0.00
30	2.0±0.17	2.0±0.20	1.0±0.30	2.0±0.20
40	2.0±0.40	2.0±0.00	2.0±0.00	2.0±0.05
Negative control (DMSO)	NA	NA	NA	NA
Positive control (Amoxicillin)	3.0±0.10	2.0±0.00	2.0±.050	3.0±0.17

Table 6: Minimum inhibitory concentration (MIC) of *Ficus species* against the different bacterial strains.

BACTERIAL STRAINS				
MIC(mg/ml)				
Plant used	***Escherichia coli* (40)**	***Salmonella typhimurium* TA (98)**	***Salmonella typhimurium* TA (100)**	***Pseudomonas aeruginosa* (424)**
Ficus religiosa	*10mg/ml*	*NA*	*10mg/ml*	*20mg/ml*
Ficus elastica	*20mg/ml*	*NA*	*10mg/ml*	*20mg/ml*
Ficus benghalensis	*NA*	*20mg/ml*	*NA*	*20mg/ml*
Ficus infectoria	*NA*	*20mg/ml*	*NA*	*NA*
Ficus benjamina	*NA*	*NA*	*NA*	*NA*
Positive control (AMXO)	0.03mg/ml	0.25mg/ml	0.25mg/ml	0.125mg/ml
Negative control (DMSO)	NA	NA	NA	NA

The order of percentage inhibition of *Ficus benghalensis* against different bacterial strains was found to be in the following order: *Salmonella typhimurium* (TA98) [45%] > *Pseudomonas aeruginosa* [42%] > *Escherichia coli* [NA] = *Salmonella typhimurium* (TA100) [NA].

The broth dilution method was used to determine the minimum inhibitory concentration (MIC) against different bacterial strains *viz*. *Salmonella typhimurium* (TA98), *Salmonella typhimurium* (TA100), *Escherichia coli* and *Pseudomonas aeruginosa*. It was observed that leaf extract of *Ficus elastica* inhibited the growth of *Salmonella typhimurium* (TA100). It was also noticed that the effect on the growth and multiplication of bacteria was dose dependent. On the contrary, the extract was found completely ineffective to inhibit the growth of *Salmonella typhimurium* (TA98) (Table 6). The order of antibacterial activity in the form of minimum inhibitory concentration of *Ficus elastica* was observed to be as follows: *Salmonella typhimurium* (TA100) [10 mg/ml] > *Pseudomonas aeruginosa* [20 mg/ml] = *Escherichia coli* [20 mg/ml] > *Salmonella typhimurium* (TA98) [40 mg/ml].

The percentage of inhibition of the methanol extract of *Ficus elastica* on the growth of different microorganisms were in the following order: *Escherichia coli* [42%] > *Salmonella typhimurium* (TA100) [40%] = *Pseudomonas aeruginosa* [40%] > *Salmonella typhimurium* (TA98) [38%].

No effect was seen in the methanol extract of leaves of *Ficus benjamina* against all the tested bacterial strains.

Table 6 indicate the effect of methanol extract of leaves of *Ficus infectoria*. It was found that the *Ficus infectoria* inhibited the growth of *Salmonella typhimurium* (TA98) and *Pseudomonas aeruginosa*. It was also noticed that the effect on the growth of bacteria was dose dependent. On the contrary, the extract was completely ineffective to control the growth of *Salmonella typhimurium* (TA100) and *Escherichia coli*. The order of antibacterial activity in the form of minimum inhibitory concentration of *Ficus infectoria* was observed to be as follows: *Salmonella typhimurium* (TA98) [20 mg/ml] > *Pseudomonas aeruginosa* [40 mg/ml] > *Escherichia coli* [NA] = *Salmonella typhimurium* (TA100) [NA].

The methanol extract of *Ficus infectoria* showed the percentage of inhibition on the growth of different microorganisms in the following order: *Pseudomonas aeruginosa* [50%] > *Salmonella typhimurium* (TA98) [42%] = *Escherichia coli* [NA] = *Salmonella typhimurium* (TA100) [NA].

Amoxicillin was used as standard drug which was found to inhibit the growth of all the tested strains at the concentration of 1, 0.5, 0.25, 0.125, 0.06, 0.03 and 0.015 mg/ml. It was also seen that the effect on the growth and multiplication of bacteria was dose dependent. It was also noticed that even at lower concentrations: 0.03 mg/ml and 0.125 mg/ml, a very good inhibitory effect was observed against *Escherichia coli* and *Pseudomonas aeruginosa* respectively. The order of antibacterial activity in the form of minimum inhibitory concentration of Amoxicillin was observed to be as follows: *Escherichia coli* [0.03 mg/ml] > *Pseudomonas aeruginosa* [0.125 mg/ml] > *Salmonella typhimurium* (TA100) [0.25 mg/ml] = *Salmonella typhimurium* (TA98) [0.25 mg/ml].

The percentage of inhibition of the amoxicillin on the growth of different micro organisms was in the following order: *Salmonella typhimurium* (TA98) [60%] > *Pseudomonas aeruginosa* [59.4%] > *Salmonella typhimurium* (TA100) [58%] = *Escherichia coli* [58%].

DISCUSSION:

Medicinal plants are rich source of secondary metabolites or phytochemicals and are responsible for various pharmacological activities. The increasing antibiotic resistance exhibited by numerous pathogen has led to the search and screening of several medicinal plants for their potential antibacterial activities so as to overcome various diseases caused by multidrug resistant pathogens.

Genus *Ficus*, being a renowned element in Ayurveda, is used in this experimentation. The antibacterial activity of the methanol extract of *Ficus religiosa*, *Ficus benghalensis*, *Ficus elastica*, *Ficus banjamina* and *Ficus infectoria* against *Salmonella typhimurium* (TA98), *Pseudomonas aeruginosa*, *Salmonella typhimurium* (TA100) and *Escherichia coli* was determined by implying disc diffusion assay. The minimum inhibitory concentrations (MIC) of the extracts was determined by broth dilution method. The tested *Ficus* species belong to family Moraceae possess antibacterial and antifungal activities against various microbial species. By reviewing the literature it was found that the methanol extracts of *F. religiosa* and *F. bengalensis* from plant barks were reported to be possessing inhibitory effects against *E. coli* isolated from diarrheal patients [5]. It was also reported that the aqueous extracts from the barks of *F. benghalensis* were reported to be possessing antimicrobial activity against *P. aeruginosa.* The presence of tannins, saponins, flavonoids, glycosides, phenolic compounds, carbohydrates and proteins make them efficient antimicrobial agents against many bacterial strains. Tannins and saponins are considered to be the major phytochemicals which shows antibacterial activities [6]. The methanolic extracts of both the plant barks were reported to be possessing inhibitory effects against *E.coli* and *P. aeruginosa* as reported by Ramakrishnaiah, 2012 [7].

Similarly, the methanolic extracts of stem, roots and leaves of *F. benjamina* were reported to have antibacterial activity. The presence of phenolic compounds and flavonoids mainly contribute the antibacterial activity against different bacterial strains [8]

The another member of this family *i.e. F. elastica* was also reported to be having inhibitory effects against Gram positive and Gram negative bacteria. The fruit latex extracts from this plant was known to be possessing antibacterial and antifungal activities due to the presence of active components such as tannins, saponins, flavonoids, phenolic compounds and enzymes [9]. Similarly, the barks extracts of *F. infectoria* were also reported to be effective against bacterial strains causing infections in humans.

It has been also reported that *Ficus* plant extracts are responsible for curing numerous diseases like respiratory diseases, sexual disorders, central nervous system (CNS), gastric problems, skin diseases and diabetes [10]. Due to its antibacterial activity, *Ficus species* were used in folk medicines as an astringent, carminative and to cure stomachic, vermonicide, hypotensive and helmentic. This plant has gained much importance in Ayurveda for preparing many medicines which cures dysentery and diarrhea. The results obtained in the present study are found to be consistent with the similar observation made by different workers in literature. The methanol extracts of leaves of *Ficus species* showed the antibacterial effects because of two reasons:

(1) The higher extraction capacity of methanol may have produced a large number of active constituents that might be the reason for antibacterial activity.
(2) The presence of biologically active components like tannin, terpenoids, alkaloids, saponins, flavonoids, essential oils and phenolic compounds, which are in high amount, are responsible for antimicrobial properties.

CONCLUSION:

The methanol extracts of the leaves of *F. religiosa, F. benghalensis, F. elastica, F. banjamina* and *F. infectoria* were used in this study. Among them the *F. benghalensis. F. religiosa* and *F. elastica* are more effective than *Ficus infectoria* and *Ficus banjamina.* Because of this reason they may be used in traditional medicines. Further, an investigation on the active components needs to be done to know the exact mechanism of action which will contribute much more to the development of advanced pharmaceuticals.

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