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Evaluation of Phytochemical Constituents and Antibacterial Activities of *Symphytum officinale* L.

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The present study was carried out on *Symphytum officinale* L. for its phytochemical and antibacterial activity by Disc diffusion method using different solvents such as petroleum ether, ethyl acetate, chloroform, methanol, acetone and aqueous. S*ymphytum officinale* L. leaf and root parts were taken in this study. Steroids, Triterpenoids, Reducing sugars, Sugars, Phenolic compounds, Flavonoids, Saponins and Tannins are present in both leaf and root extract of *Symphytum officinale*. The antibacterial activity of chloroform leaf extract of *Symphytum officinale* showed best results against *Bacillus cereus*. The antibacterial activity of the root extract of *Symphytum officinale* were studied by Disc diffusion method and it was found that methanol extract showed maximum inhibitory effect against the bacterium *Proteus vulgaris* and *Staphylococcus aureus*.

Key words: *Symphytum officinale* L., Antibacterial activity, Phytochemical Analysis.

Plants are a tremendous source for the discovery of new products of medicinal value for drug development. Today several distinct chemicals derived from plants are important drugs currently used in most of the countries in the world. Since time immemorial nature's own supreme creation, man has been completely dependent on plants and as civilization developed he has learned to exploit natural resources and to make use of every bit of it. Sato *et al*., (1997). Primitive man tried to cure diseases from plants growing abundantly around him. His experience through trial had taught him a lot about the medicinal properties of different plants. But at a practical level, only those plants whose medicinal use has already been discovered for human or veterinary application are considered medicinal. There are estimated to be around 9000 species of medicinal plants that are used in different systems of Indian medicine (Clark, 1996).

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria

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have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen M.L). Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. (Ramadevi, 1998).

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. (Perumalsamy & Ignacimuthu 2000).

MATERIAL AND METHODS

Collection of plant

The plant materials were collected from the Tiruchirappalli District, Tamil Nadu, India . *Symphytum officinale* L. It is commonly known as Comfrey.Symphytum, healing herb, knitback, ass-ear, back wort, blackwort, bruisewort, gum plant, slippery-rootCommon Comfrey. Knitbone. Knitback. Consound. Blackwort. Bruisewort. Slippery Root. Boneset. Yalluc (Saxon). Gum Plant. Consolida. Ass Ear.

Medicinal properties

Traditionally, its roots and leaves have been used to treat broken bones and wounds. The mucilaginous root content was formerly promoted as an expectorant and antitussive, and to treat gastrointestinal disorders. Comfrey is promoted in Ayurvedic and other herbal systems, with claims for benefit in disorders such as peptic ulcer. Comfrey also has been commonly used as a topical anti inflammatory healing agent.

Phytochemical Analysis

The roots and leaves of the plant were taken for the present study. 500 mg of plant materials were collected, cut into small pieces and shade dried under controlled condition. Each dry plant material was taken for extraction in the soxhlet apparatus using the various solvents such as petroleum ether, ethyl acetate, chloroform, methanol, acetone and aqueous.

Qualitative analysis

All the six extracts (leaves and roots) obtained were used to carry out qualitative analysis as per Brindha *et al*., (1981) method in order to partially identify the compounds that may be present in the corresponding extract.

Extraction of plant material

The shade dried plant materials were weighed and taken in the soxhlet apparatus with the particular solvent. The extraction was done for 72 hours. The extract was then condensed and kept in separate vessel. The above procedure was carried out simultaneously using other solvents.

Disc preparation

The disc with 6mm diameter was prepared from whatmann no.1 filter paper. The discs were sterilized by autoclave at 12°C. After the sterilization the moisture disc were on hot air oven at 50°C.The various solvent extracts disc and control discs were prepared.

Screening of antibacterial activity

The antibacterial activity of *Symphytum officinale* leaf and root (Petroleum ether, Ethyl acetate, Chloroform, Methanol, Acetone and Aqueous) extracts against the various bacteria namely *E. coli, Klebsiella pneumoniae, Enterobacter aerogens, Salmonella typhi, Proteus vulgaris* and *Pseudomonas aeruginosa* (gram negative), *Staphylococcus aureus* and *Bacillus cereus* (gram positive) was examined in the present study and their potency was assessed by the presence and absence of inhibition zone and its diameter by disc diffusion method. The results indicate that organic extracts exhibited greater antibacterial activity than aqueous extract. This can be attributed to the presence of antibacterial active principles which are either polar or non-polar.

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 used and also between the lengths of incubation

RESULTS AND DISCUSSION

The phytochemical study and antibacterial screening of the leaf and root extract

of *Symphytum officinale* was carried out in the present study. Antibacterial activity of Petroleum ether, Ethyl acetate, Chloroform, Methanol, Acetone and Aqueous leaf and root extracts of *Symphytum officinale* was carried out against eight different gram negative and gram positive bacteria standard disc diffusion method . The plant extracts were subjected to qualitative analysis proposed by Brindha *et al*., (1981). The leaf extract of *Symphytum officinale* showed the presence of steroids, Triterpenoids, sugars, Reducing sugars, phenolic compounds, flavonoids, saponins and tannins. The root extract of *Symphytum officinale* showed the presence of steroids, triterpenoids, reducing sugars, sugars, phenolic compounds, flavaonoids, tannins, saponins

Phytochemical analysis

The preliminary phytochemical analysis was carried out in the leaf and root extracts of *Symphytum officinale*. Steroids,Triterpenoids, Reducing sugars, Sugars, Phenolic compounds, Flavonoids, Saponins and Tannins are commonly present in leaf extract of *Symphytum officinale* (Table 1). Alcoholics, phenolic compounds,

Table 1. The preliminary phytochemical studies on leaf extract of *Symphytum officinale* L.

S. No.	Phytocompounds	Petroleum ether	Ethyl acetate	Chloroform	Methanol	Acetone	Aqueous
1.	Steroids	+	+	+	+	+	–
2.	Triterpenoids	+	+	+	+	–	–
3.	Reducing sugars	+	–	–	–	+	–
4.	Sugars	+	+	+	+	+	–
5.	Alkaloids	–	–	–	–	–	–
6.	Phenolic compounds	–	+	+	+	+	+
7.	Catachins	–	–	–	–	–	–
8.	Flavonoids	+	–	–	–	+	–
9.	Saponins	+	+	+	–	–	–
10.	Tannins	+	–	–	–	+	–
11.	Anthroquinones	–	–	–	–	–	–
12.	Amino acids	–	–	–	–	–	–

+ Present; – Absent

Table 2. The preliminary phytochemical studies on root extract of *Symphytum officinale* L.

S. No.	Phytocompounds	Petroleum ether	Ethyl acetate	Chloroform	Methanol	Acetone	Aqueous
1.	Steroids	+	+	+	-	+	+
2.	Triterpenoids	+	+	+	+	-	+
3.	Reducing sugars	+	-	-	-	+	-
4.	Sugars	-	-	-	+	+	-
5.	Alkaloids	-	-	-	-	-	-
6.	Phenolic compounds	-	-	-	-	+	-
7.	Catachins	-	-	-	-	-	-
8.	Flavonoids	+	-	-	-	-	-
9.	Saponins	-	-	+	-	-	-
10.	Tannins	-	-	-	-	+	-
11.	Anthroquinones	-	-	-	-	-	-
12.	Amino acids	-	-	-	-	-	-

+ Present; – Absent

Catachins, Anthroquinones, Amino acids are not found to be petroleum ether leaf extract of *Symphytum officinale* L.

Reducing sugar, alkaloids, Catachins, Flavonoids, Tannins, Anthroquinones, Amino acids are absence in ethyl acteate, chloroform, and methanol leaf extract. Steriods. Reducing sugar, sugar, phenolic compounds, Flavonoids, Tannins are showed in acetone leaf extract. Only phenolic compound are present in aquevous leaf extract (Table 1).

Steroids, Triterpenoids, Reducing sugars, Phenolic compounds, Flavonoids, Saponins and Tannins are commonly showed in all root extract (Table 2). Reducing sugar, sugar, Alkaloids, Phenolic compounds, Catachins, Flavonoids, Saponins, Tannins, Anthroquinones, Amino acids are not found to present in Petroleum ether, Ethyl acetate, Chloroform, Methanol, Acetone, Aqueous root extract of *Symphytum officinale* L.

Antibacterial activity

The antibacterial activity of *Symphytum officinale* leaf and root (Petroleum ether, Ethyl acetate, Chloroform, Methanol, Acetone and Aqueous) extracts against various bacteria namely *E. coli, Klebsiella pneumoniae, Enterobacter aerogens, Salmonella typhi, Proteus vulgaris* and *Pseudomonas aeruginosa* (gram negative), *Staphylococcus aureus* and *Bacillus cereus* (gram positive) were examined in the present study and their potency was assessed by the presence and absence of inhibition zone and its diameter by disc diffusion method. The results indicate that organic extracts exhibited greater antibacterial activity than aqueous extract. This can be attributed to the presence of antibacterial active principles which are either polar or non-polar.

The results indicated that the chloroform Leaf extract followed by Ethyl acetate extract showed considerably more inhibition activity than petroleum ether and Methanol extract. Less inhibition was noticed in the petroleum ether and Methanol extracts. In all the cases the standard antibiotic streptomycin showed greater inhibitory activity. The chloroform extract showed maximum inhibition against *Staphylococcus aureus* and *Klebsiellla pneumoniae* The mean and standard error of the diameter of the inhibition zone is determined (1.28±0.02, 1.16±0.027). Moderate inhibitory effect was observed in *Salmonella typhi* and *E. coli*. Less inhibition was observed in the case of *Enterobacter aerogens*. Ethyl acetate extract showed maximum inhibitory activity against *Proteus vulgaris* and *Salmonella typhi* (1.16±0.07, 1±0) and moderate activity against *Staphylococcus aureus* and *E. coli*. The diameter of zone revealed that petroleum ether and methanol extracts showed less inhibitory activity against bacteria and no inhibition was observed in acetone and aqueous extracts (Table 3).

The root extract (Petroleum ether, Ethyl acetate, Chloroform, methanol, acetone and aqueous of *Symphytum officinale* L. was tested against eight bacteria. The results revealed that all the six extracts were effective against the bacterial growth. Among the six extracts, methanol and acetone showed the greater activity. The moderate growth inhibitory activity was noticed in ethyl acetate and aqueous. Less inhibition was observed in petroleum ether extract. The mean and standard error indicated that the methanol extract showed greatest inhibitory effect against *Proteus vulgaris* and *Staphylococcus aureus* (2.18±0.049, 1.73±0.05 respectively). Acetone extract showed its maximum inhibition against *S. aureus* (2.13±0.108) and chloroform extract against *Bacillus cereus* (1.7±0.0). Ethyl acetate extract showed the inhibitory activity in its maximum against *Pseudomonas aeruginosa.* The maximum inhibitory activity of aqueous extract was against *Pseudomonas aeruginosa* and *Bacillus cereus* (Table 4). Similar results have been obtained by Oguti *et al*., (1991) who worked on the ethonolic n-Hexane.Choloroform, ethyl acetate and methnolic extracts of *Cassia alata* and 16 fractions were assayed for antibacterial and antifungal activity. According to Saxena *et al*.,(1994). The methyl ester of 3,4,5-trihydroxy benzoic acid(methyl gallate) 4 methoxy and 3,5-di hydroxy benzoic acid and gallic acid in has glabra are responsible for antibacterial activity. Erasto *et al*.,(2004) studied antimicrobial activity and antioxidant effect of flavonoids 5,7,4-trihydroxy -4-methoxy isoflave-3ene, 6'6-di hydroxyl-4 methoxy-2-aryl benzo-furan in addition to 8 flavonoids, derron, medicarpan, genstein, weighteone, lupiweighteone, gancwoin C,7-hydroxy 4 methoxy isoflavone and 7,3 dihydroxy 4 methoxy isoflavone were isolated from the root of Bolusanthus speciosus (Table 4).

The antibacterial activity of leaf extract of *Symphytum officinale* were determined for each

Table 3. Antibacterial screening of leaf extract of *Symphytum officinale* L. by disc diffusion method

S. No.	Bacterial strains	Petroleum Ether Mean ± SE	Ethyl acetate Mean ± SE	Chloroform Mean ± SE	Methanol Mean ± SE	Acetone Me an ± SE	Aqueous Mean ± SE	Standard antibiotic 30 µl/disc
1.	*Escherichia coli*	0.76±0.027	0.73±0.054	1.00±0	0.9+0	0.8+0	–	2.63±0.054
2.	*Klebsiella pneumoniae*	0.73±0.054	–	1.12±0.047	–	–	–	2.63±0.054
3.	*Enterobacter aerogens*	0.76±0.027	0.76±0.027	0.83±0.027	0.8+0	0.8+0	–	2.63±0.054
4.	*Salmonella typhi*	0.86±0.027	1.00±0	0.86±0.027	–	–	–	2.36±0.027
5.	*Proteus vulgaris*	0.85±0.023	1.28±0.02	–	–	–	–	2.4±0.0
6.	*Pseudomonas aeruginosa*	–	–	–	–	–	–	2.4±0.0
7.	*Staphylococcus aureus*	0.83±0.015	–	1.28±0.07	–	0.89±0.023	–	2.4±0.0
8.	*Bacillus cereus*	1.16±0.070	–	1.16±0.070	–	0.82±0.011	–	2.4±0.0

Table 4. Antibacterial screening of root extract of *Symphytum officinale* L. by disc diffusion method (Zone diameter in cm)

S. No.	Bacterial strains	Petroleum Ether Mean ± SE	Ethyl acetate Mean ± SE	Chloroform Mean ± SE	Methanol Mean ± SE	Acetone Me an ± SE	Aqueous Mean ± SE	Standard antibiotic 30 µl/disc
1.	*Escherichia coli*	-	0.71 0.04	-	1.02 0.70	1.23 0.0	-	-
2.	*Klebsiella pneumoniae*	-	-	-	-	-	0.85 0.018	-
3.	*Enterobacter aerogens*	0.71 0.027	0.82 0.02	-	1.21 0.071	-	0.87 0.005	-
4.	*Salmonella typhi*	0.91 0.027	0.82 0.02	0.90 0.027	1.50 0.110	1.18 0.0	-	-
5.	*Proteus vulgaris*	0.9 0	0.94 0.01	0.85 0.027	2.18 0.049	-	0.89 0.041	2.53 0.014
6.	*Pseudomonas aeruginosa*	1.16 0.023	1.31 0.07	0.85 0.027	1.20 0.091	-	1.30 0	2.53 0.014
7.	*Staphylococcus aureus*	0.87 0	-	0.67 0.027	1.73 0.05	1.54 0.0	1.23 0.010	-
8.	*Bacillus cereus*	0.8 0	0.92 0.02	1.7 0	1.38 0.020	1.54 0.0	1.28 0.05	2.53 0.014

leaf extract of *Symphytum officinale* L. and chloroform leaf extract showed the best result was against *B.cereus* (Table 3).

The antibacterial activity of the root extract of *Symphytum officinale* L. was studied by the disc diffusion method and it was found that methonal extract showed maximum inhibitory effect and the bacterium *Proteus vulgaris* and *Staphylococcus aureus* were susceptible to it (Table 4).

CONCLUSION

The phytochemical study and antimicrobial screening of the leaf and root extract of *Symphytum officinale* L. was carried out in the present study. From the above studies, it is concluded that the traditional plants may represent new sources of anti-microbials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. It can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

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