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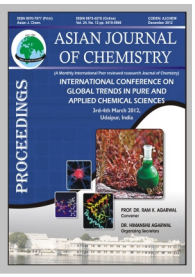

Phytochemical Screening and Evaluation of Biological Activities of Some Medicinal Plants of Phagwara, Punjab†

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In the present work, phytochemical screening of 12 medicinal plant species namely *Ocimum sanctum, Psoralea corylifolia, Crotolarea juncea, Sansevieria ghiana, Gossypium herbaceum, Schleichera oleosa, Anethum sowa, Analrographis panicculata, Punica granatum, Argemone mexicana, Gmelina arborea* and *Prunella vulgaris* has been carried out. Saponins isolated from *O. sanctum* and *A. mexicana* have shown excellent antibacterial potential against *E. coli* with inhibition zone of 30 and 28 mm respectively. Alkaloids from *P. corylifolia, G. herbaceum, S. oleosa, S. ghiana, P. granatum* and *C. juncea* possess potential activity against *E. coli*. Flavonoids of *O. sanctum, P. corylifolia* and *P. granatum* possess significant potential against *E. coli*. These studies will be helpful in developing plant based antimicrobial formulations, which are expected to be superior to synthetic antimicrobial drugs and formulations.

Key Words: Alkaloids, Saponin, Flavonoids, Antibacterial activity, *Ocimum sanctum, Argemone mexicana, Punica granatum, Psoralea corylifolia, Gossypium herbaceum, Schleichera oleosa*.

INTRODUCTION

Nature has provided many things for humankind over the years, including the tools for the first attempts at therapeutic intervention. Ancient civilization depended on plant extracts for the treatment of various ailments. Today, plant materials remain an important resource for combating illnesses, including infectious diseases and many of these plants have been investigated for novel drugs or used as templates for the development of new therapeutic agents, food additives, agrochemicals and industrial chemicals[1]. Plant based natural constituents can be derived from different parts of the plant like bark, leaves, flowers, roots, fruits, seeds, *etc*. The phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as an defense system against disease or more accurately, to protect against disease. Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds and many more such as flavonoids and tannins *etc*.[2].

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. But among the 250,000-500,000 plant species only a small percentage has been investigated phytochemically[3]. So the systematic screening of plant species with the purpose of discovering new bioactive compounds can help us to cure many fungal and bacterial diseases of economically important crops. The plant chemicals have been found to possess biocidal activity against several pests and pathogens[4-6]. These are superior to synthetic pesticides in a number of ways like low mammalian toxicity, target specificity and biodegradability.

EXPERIMENTAL

Collection of plant material: On the bases of ethnobotanical knowledge of available literature and visual observations of plants that were relatively free from diseases and insect damages 12 plant species have been selected for the present study. Collected plant material were thoroughly washed and then dried under shade at 25 ± 2 ºC for about 10 days. The dried plant samples were ground well into a fine powder in a mixer grinder. The powdered samples were then stored in air tight containers at room temperature.

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TABLE-1
PHYTOCHEMICAL SCREENING

Name of plant species	Chemical constituents							
	Alkaloid	Flavanoid	Saponin	Terpenoid	Steroid	Tannin	Cardiac glycoside	Phlobatannin
Andrographis paniculata	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve
Anethum sowa	+ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
Argemone mexicana	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve
Crotolarea juncea	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Gmelina arborea	-ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve
Gossypium herbaceum	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve
Ocimum sanctum	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve
Prunella vulgaris	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve
Psoralea corylifolia	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
Punica granatum	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve
Sansevieria ghiana	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
Schleichera oleosa	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve

Phytochemical screening: Phytochemical screening has been carried out following the procedures reported in literature[7,8].

Qualitative analysis on phytochemical constituents

Test for tannins: Powdered sample (0.5 g) of each plant is boiled in 20 mL of distilled water in a test tube and then filtered. The filtration method used here is the normal method, which includes a conical flask and filter paper. 0.1 % $FeCl_3$ is added to the filtered samples and observed for brownish green or a blue black colouration, which shows the presence of tannins.

Test for phlobatannins: In a test tube take 10 mL of aqueous extract of each plant sample and boiled it with 1 % HCl. If the sample of plant carries phlobatannins, a deposition of a red precipitate will occur and indicates the presence of phlobatannins.

Test for saponins: Two g of powdered samples of each plant is boiled together with 20 mL of distilled water in a water bath and filtered. Ten mL of the filtered sample is mixed with 5 mL of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing is then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicates the presence of saponins.

Test for flavonoids: A few drops of 1 % NH_3 solution is added to the aqueous extract of each plant sample in a test tube. A yellow colouration is observed if flavonoid compounds are present.

Test for terpenoids: Five mL of aqueous extract of each plant sample is mixed with 2 mL of $CHCl_3$ in a test tube. Three mL of concentrated H_2SO_4 is carefully added to the mixture to form a layer. An interface with a reddish brown colouration is formed if terpenoids constituent is present.

Test for cardiac glycosides: One mL of concentrated H_2SO_4 is prepared in a test tube. 5 mL of aqueous extract from each plant sample is mixed with 2 mL of glacial CH_3CO_2H containing 1 drop of $FeCl_3$. The above mixture is carefully added to the 1 mL of concentrated H_2SO_4 so that the concentrated H_2SO_4 is underneath the mixture. If cardiac glycoside is present in the sample, a brown ring will appear indicating the presence of the cardiac glycoside constituent.

Quantitative analysis on phytochemical constituents

Alkaloids: Five g of the plant sample is prepared in a beaker and 200 mL of 10 % CH_3CO_2H in C_2H_5OH is added to the plant sample. The mixture is covered and allowed to stand for 4 h. The mixture then filtered and the extract is allowed to become concentrated in a water bath until it reaches 1/4 of the original volume. Concentrated NH_4OH is added until the precipitation is complete. The whole solution is allowed to settle and the precipitate is collected and washed with dilute NH_4OH and then filtered. The residue is alkaloid, which is then dried and weighed.

Saponins: The samples were ground and 20 g of each plant sample is put into a conical flask and 100 mL of 20 % C_2H_5OH is added to the plant sample. The sample is heated over a hot water bath for 4 h with continuous stirring at about 55 ºC. The mixture is then filtered and the residue re-extracted with another 200 mL of 20 % C_2H_5OH. The combined extracts are reduced to 40 mL over a water bath at about 90 ºC. The concentrated is then transferred into a 250 mL separator funnel and 20 mL of $(CH_3CH_2)_2O$ is added to the extract and shaken vigorously. The aqueous layer is recovered while the $(CH_3CH_2)_2O$ layer is discarded and the purification process is repeated. 60 mL of n-C_4H_9OH is added and the combined n-C_4H_9OH extracts is washed twice with 10 mL of 5 % NaCl. The remaining solution is then heated in a water bath and after evaporation; the samples are dried in the oven to a constant weight.

Flavonoids: Ten g of plant sample is repeatedly extracted with 100 mL of 80 % aqueous methanol at room temperature. The whole solution is then filtered through filter paper and the filtrate is later on transferred into a water bath and solution is evaporated into dryness. The sample is then weighed until a constant weight.

Screening of chemical constituents for antibacterial activity: Molten nutrient agar medium was poured in sterilized petriplates and allowed to come at room temperature. 100 µL of bacterial culture in nutrient agar was added to petriplates. After solidification one sterilized paper disc loaded with 20 µL (equivalent to 0.02 mg of chemical constituent) plant constituent and another with pure solvent were placed in petriplates and kept in incubator at 37 ± 1 ºC. Inhibition zones were measured in mm after 36 h. of incubation. The treatments were replicated three times in randomized block design[9].

RESULTS AND DISCUSSION

Phytochemical screening of collected plant species has been carried out following the methods reported in literature and the results have been reported in Table-1.

All the plant species except *Andrographis paniculata* and *Gmelina arborea* chosen for the present study have shown the presence of alkaloids, while flavonoids have been found to be absent in *Gossypium herbaceum, Schleichera oleosa, Prunella vulgaris* and *Sansevieria ghiana*. Saponins have been found to be present in all species except *Crotolarea juncea, Andrographis paniculata, Prunella vulgaris, Anethum sowa and Psoralea corylifolia*. These three active constituents of plant species possess maximum antimicrobial activity. Terpenoids have been found to be absent in *Schleichera oleosa, Punica granatum, Andrographis paniculata* and *Crotolarea juncea*. Steroids and cardiac glycosides have been found to be present in plant species namely *Psoralea corylifolia, Crotolarea juncea* and *Gmelina arborea*. Phlobatannins have been found to be present in four species namely *Ocimum sanctum, Gossypium herbaceum, Punica granatum* and *Andrographis paniculata*.

Quantitative estimation of alkaloids, saponins and flavonoids: The quantitative estimation of alkaloid and saponin contents of different plant species has been undertaken as per methods reported in literature and the results have been reported in Table-2. *Ocimum sanctum* has been found to possess 32.8 % saponin content followed by *Gossypium herbaceum*. *Gmelina arborea* possess 15.2 % flavonoid, followed by *Psoralea coryliofolia*.

TABLE-2
QUANTITATIVE ESTIMATION OF ALKALOIDS, SAPONIN AND FLAVONOIDS

Name of plant species	Alkaloid content (w/w %)	Saponin content (w/w %)	Flavanoid content (w/w %)
Andrographis paniculata	-	8.53	-
Anethum sowa	5.44	-	6.02
Argemone mexicana	5.67	1.75	-
Crotolarea juncea	4.26	-	6.24
Gmelina arborea	-	6.1	15.2
Gossypium herbaceum	5.40	29.7	-
Ocimum sanctum	5.92	32.8	7.38
Prunella vulgaris	5.75	-	-
Psoralea corylifolia	4.82	-	8.14
Punica granatum	4.62	8.50	7.77
Sansevieria ghiana	2.81	-	5.64
Schleichera oleosa	4.83	9.1	-

Alkaloids separated from *Psoralea corylifolia, Sansevieria ghiana, Gossypium herbaceum, Schleichera oleosa, Andrographis paniculata* and *Punica granatum* have shown potential activity against *E. coli*. Alkaloids from *Psoralea corylifolia* has shown maximum activity of 14 mm followed by that of *Sansevieria ghiana*, *Gossypium herbaceum, Schleichera oleosa, Andrographis paniculata*, *Punica granatum*.

Saponins isolated from *Ocimum sanctum* have exhibited maximum inhibition zone of 30 mm followed by *Argemone Mexicana*, *Schleichera oleosa* and *Gossypium herbaceum* (Table-3).

TABLE-3
ANTIBACTERIAL POTENTIAL OF ALKALOIDS, SAPONINS AND FLAVONOIDS ISOLATED FROM PLANT SPECIES AGAINST *E. coli*

Name of plant species	Alkaloid (inhibition zone in mm)	Saponin (inhibition zone in mm)	Flavanoids (inhibition zone in mm)
Andrographis paniculata	-	6	-
Anethum sowa	0	-	0
Argemone mexicana	0	28	0
Crotolarea juncea	11	-	0
Gmelina arborea	-	0	0
Gossypium herbaceum	12	8	-
Ocimum sanctum	0	30	8
Prunella vulgaris	0	-	-
Psoralea corylifolia	14	-	18
Punica granatum	11	0	13
Sansevieria ghiana	12	-	0
Schleichera oleosa	12	9	-

Flavanoids isolated from *Psoralea corylifolia, Punica granatum, Gmelina arborea* have shown potential activity against *E. coli.* Flavanoids of *Psoralea corylifolia* has shown maximum activity with inhibition zone of 18 mm against *E. coli.*

Plant derived antimicrobial formulations are believed to be superior to their synthetic counterparts as these have low mammalian toxicity, target specificity and biodegradability. Plant based formulations may possess many active ingredients although in low concentration. Thus, the pathogens do not develop resistance to them, because it requires several simultaneous mutations to occur in pathogens' genetic constituents to overcome all the ingredients of the plant based formulations. These results will be helpful in development of antibacterial formulations.

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