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Bioprospecting of selected folkloric medicinal plants that ameliorate the microbial infections

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In this study, four plants were taken namely, *Blepharis maderaspatensis, Solanum nigrum, Acalypha indica*, and *Tridax procumbens* which are widely reported in the folkloric medicinal system for curing wounds and microbial borne skin infections. The drug principle has been extracted from different plant parts of the selected plants using six solvent systems ranging from non-polar to polar, namely, petroleum ether, benzene, chloroform, acetone, ethanol and methanol. The best extraction system has been standardized and the best solvent identified using different solvents by employing the principle of solid-liquid extraction using two methods, namely, hot continuous (Soxhlet) successive extraction and cold extraction (Maceration). The efficiency of the successive method has been compared with cold extraction (Maceration). The qualitative phytochemical constituents in the solvent extracts have also been analyzed. Thereby, we determine that the composition of the herbal extracts that depends on the type of the solvent system has been determined and so does the temperature employed in the solvent extraction.

Key words: Successive extraction, hot extraction, cold extraction, phytochemicals, drug principles, *Blepharis maderaspatensis, Solanum nigrum, Acalypha indica*, *Tridax procumbens*.

INTRODUCTION

The usage of natural herbs as drugs has been very minimal for various reasons of which the following play a greater role: the less availability of herbs, the high prices of rare medicinal species and difficult cultivating conditions. However, the indiscriminate use of antibiotics and the blind dependence on synthetics has become outdated and people are returning to the naturals with a hope to surmount exacerbate antibiotic resistance by the pathogenic microbes (Dancey and Chen, 2006; Khan, 2006; Marjorie Murphy Cowan, 1999). This situation has forced scientists to search for new antimicrobial substances from various natural sources like herbs/medicinal plants and marine sources (Harsha et al., 2003).

Traditional medicines using plant extracts continue to provide health coverage for over 80% of the world's population, especially in the developing countries (WHO, 2002). Even today, tribals and certain local communities in India still practice herbal medicine to cure a variety of skin diseases and disorders (Parinitha Mahishi et al., 2005). The efficacy of the plants in curing various skin infections has been well established and a large volume of work has been done in this field by researchers in India and abroad (Ignacimuthu et al., 2006). Here, we are the first to report the antimicrobial activity and phytochemical compounds of the folkloric medicinal plant *Blepharis maderaspatensis*.

In this study, we have chosen the four plants mentioned in the folkloric medicinal system that ameliorate the skin infections, namely, *B. maderaspatensis, Solanum nigrum, Acalypha indica* and *Tridax procumbens*. The efficient solvent system for the extraction of the drug principles from these plant parts were found

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by using a range of non-polar to polar solvents for extracting the drug principles, namely, petroleum ether, benzene, chloroform, acetone, ethanol, and methanol.

To analyze the antimicrobial activity of the respective solvent extracts the efficiency of the successive method has been compared with cold extraction (Maceration). The qualitative phytochemical constituents in these solvent extracts were respectively analyzed; also, the composition of the herbal extracts that depends on the type of the solvent system and the temperature employed in the solvent extraction were determined.

MATERIALS AND METHODS

Chemicals

All solvents of analytical grade were used: petroleum ether, benzene, chloroform, acetone, ethanol, and methanol were obtained from Qualigens. Concentrated hydrochloric acid, glacial acetic acid, toluene, ethyl acetate, concentrated sulphuric acid, and nitric acid were obtained from SD Fine. Mueller-Hinton agar, nutrient agar, Agar-Agar and ferric chloride were purchased from Himedia.

Plant

The plant materials used for this study are as follows:

1) *B. maderaspatensis* (Family: Acanthaceae); parts used: leaf.
2) *S. nigrum* (Family: Solanaceae); parts used: leaf and stem.
3) *A. indica* (Family: Euphorbiaceae); parts used: leaf, stem, and root.
4) *T. procumbens* (Family: Asteraceae); parts used: leaf and flower.

The plants has been authenticated by the Botanical Survey of India, Coimbatore, the respective herbarium number were given in the parenthesis *B. maderaspatensis* (BSC/SC/5/23/09-10/Tech-1697) collected from Pannaipuram, Theni district, Tamilnadu, India, *A. indica (*BSC/SC/5/23/10-11/Tech-47) collected from Pannaipuram, Theni district, Tamilnadu, India. *S. nigrum* (BSC/SC/5/23/10-11/Tech-45) collected from Siruvani, Coimbatore district, Tamilnadu, India, and *T. procumbens* (BSC/SC/5/23/10-11/Tech-46.) collected from Siruvani, Coimbatore district, Tamilnadu, India. The plant samples were washed thoroughly in tap water and then with distilled water and were kept for drying. Dried plant parts were taken separately and powdered for extraction.

Active compound extraction

Two extraction methods, hot continuous (Soxhlet) successive extraction and cold extraction (Maceration) were carried out to elucidate the active drug principle from selected medicinal plants.

Test organisms

The bacterial strains used in this study were obtained from MTCC, Chandigarh, India. *Staphylococcus aureus* (MTCC code-3381), *Streptococcus mutants* (MTCC code-497), *Lactobacillus acidophilus* (MTCC code- 447), *Shigella sonnei* (MTCC code-2957), *Salmonella paratyphi* (MTCC code-3220), and *Klebsiella pneumonia (*MTCC code-3384) strains were subcultured and grown in nutrient broth at suitable temperatures.

Qualitative phytochemical screening of the plant extracts

Preliminary qualitative phytochemical screening of the solvent extracts of plant parts for phenols, sterols, proteins, resins, steroids, tannins, glycosides, terpenoids, reducing sugars, pholabatamins were performed to detect the chemical constituents by following the standard protocols (Trease and Evans, 2002; Kokate, 1994; Trease and Evans, 1989; Mace, 1963; Pew, 1948; Shinoda, 1928).

Ferric chloride test for phenols

The extract (50 mg) was dissolved in 5 ml of distilled water. To this, a few drops of neutral 5% ferric chloride solution were added. A dark green colour indicates the presence of phenolic compounds.

Liebermann-Buchard test for sterols

The insoluble residue was dissolved in chloroform and a few drops of acetic anhydride were added along with a few drops of concentrated sulphuric acid from the sides of the test tube and was observed for the formation of blue to blood red colour.

Liebermann-Buchard test for phlobatannins

A few drops of this extract was taken in boiling tube and boiled with 1% aqueous hydrochloric acid and then was allowed to stand to develop a red precipitate.

Xanthoprotein test for proteins

To 1 ml of this extract, a few drops of nitric acid was added by the sides of the test tube and observed for formation of yellow colour.

Xanthoprotein test for resins

Distilled water of 5 ml was added to the extract and observed for turbidity.

Xanthoprotein test for steroids

Acetic anhydrides of 2 ml was added to 0.5 g of this extract and 2 ml of concentrated sulphuric acid were added by the sides of the test tube and was observed for the colour change from violet to blue-green.

Xanthoprotein test for tannins

About 0.5 g of each of the extract was taken in a boiling tube and was boiled with 20 ml distilled water and then filtered. To the filtrate, a few drops of 0.1% ferric chloride were added and was well mixed and allowed to stand for some time, and was later observed for brownish green or a blue-black colouration.

Keller-Killani test for glycosides

Glacial acetic acid of 1 ml containing traces of ferric chloride and 1 ml of concentrated sulphuric acid were added to 0.5 ml of this extract and was observed for the formation of reddish brown colour at the junction of two layers and the upper layer turned bluish green in the presence of glycosides.

Acidic compounds

To the alcoholic extract, sodium bicarbonate solution was added and observed for the production of effervescences.

Salkowski test for terpenoids

To 0.5 g of this extract, 2 ml of chloroform and concentrated H_2So_4 (3 ml) were carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Fehling's reagent test for reducing sugar

A few drops of Fehling's solution A and B were added in equal volume to the dilute extracts, and were heated for 30 min and later observed for the formation of brick red coloured precipitate.

Antimicrobial assay by well diffusion assay

Antimicrobial activity of the plant's leaf and flower extracts was examined by well diffusion technique against the test organisms. Log phase cultures of test organisms on nutrient broth were seeded by spread-plate method on Mueller-Hinton agar. Approximately six wells of uniform size (0.65 cm) were made with a cork-borer onto the plates inoculated with test organisms, each well being placed at 3 cm apart. Crude plant extracts of 50 µl were respectively added into the well aseptically. Control wells with 50 µl of the pure solvents were also made on the respective plates and were incubated at 37°C for 24 h. Commercial antibiotics were taken as positive control.

RESULTS AND DISCUSSION

The anti-microbial activity of the plant extracts against test pathogens are tabulated in Table 1. The authenticity of the antimicrobial activity exhibited by all the extracts in comparison to the commercially available broad spectrum antibiotics, namely, imipenem and chloramphenicol were checked. The zones of inhibition exhibited by the test pathogens are represented in Table 2. Imipenem controls all the three Gram negative test pathogens, namely, *S. sonnei*, *S. paratyphi* and *K. pneumonia* and it also controls the growth of the Gram positive pathogens *S. aureus* and *L. acidophilus*. The chloramphenicol controls the Gram positive *L. acidophilus* and Gram negative *S. paratyphi*. The zones of inhibition less than 15 mm are represented by (+), zones of inhibition from 16 to 20 mm are represented by (++), and the zones of inhibition above 20 mm are indicated by (+++). The extracts that exhibited the zones of inhibition above 20 mm is taken equivalent to the activity of the broad spectrum antibiotics.

The findings of the experiments are interpreted in two different perspectives. Initially, an analysis of the solvent system was done. In this analysis, the influence of temperature in extraction process was elucidated. The phytochemical constituent in the best extract that gives good antimicrobial activity was also analyzed.

While analyzing the polarity-wise solvent extraction, acetone extracts of all plant parts except *S. nigrum* stem acetone extract, exhibited very good control of all the test pathogens. This shows that acetone is a good solvent that widely extracts the drug principles from almost all the folkloric plants. An interesting finding in the acetone solvent extraction is that; *B. maderaspatensis* leaf cold acetone extract exhibited a significant control over the test pathogens. The hot acetone leaf extract of the same plant showed no antimicrobial effect. A similar kind of behavior was exhibited by the *T. procumbens* leaf hot and cold acetone extracts. In general, the cold extracts show more activity in comparison to their corresponding hot extracts. This gives an inference that some constituents of phytochemicals may be heat labile that may lose its activity upon hot extraction protocols. Otherwise, some other phyto-chemical components which produce synergistic activity found along with the drug principle may be lost upon hot extraction.

On the other hand, using the same acetone extracts of *A. indica* leaf hot and cold extract exhibited almost same amount of antimicrobial activity. This finding, hence, gives an inference that not all the drug principles are heat labile and indicates that the hot extraction is also a good method of extraction in isolating the drug principle from some plant parts.

Ethanolic extracts also exhibited a significant control of the test organisms in comparison with the commercial antibiotics, it is significant to note that while comparing antimicrobial activity of the *A. indica* leaf hot and cold extract of ethanol, hot extract exhibits higher zone of inhibition corresponding to the respective cold extract. This indicates that certain drug molecules in *A. indica* leaf are slowly extracted by ethanol cold extraction, while the same drug molecule is exhaustively extracted only by hot extraction. The finding here is that the extraction of drug molecule can be considerably improved by raising the temperature and the composition of the herbal extracts depends on the type of the solvent system and the temperature employed in the extraction.

On observing the antimicrobial activity of the methanolic extracts, almost all showed good inhibition. Upon comparing the hot and cold extracts of *B. maderaspatensis* leaf, hot extracts completely lost its antimicrobial activity, whereas the cold extract had significant antimicrobial activity. This again supports the finding that, the temperature in the hot extraction may be responsible for the loss of the drug principle's activity that may be heat labile.

Among the petroleum ether extracts (the non-polar solvent), *A. indica* root extract exhibited antimicrobial activity against Gram negative bacteria to a greater extent than the Gram positive bacteria. This gives an indication that certain hydrophobic drug principle controls Gram negative bacteria to a greater extent than the Gram positive bacteria that can be extracted by petroleum ether.

Among the benzene extracts, *A. indica* root extract exhibited antimicrobial activity that controls Gram positive

Table 1. Antimicrobial activity represented in zone of inhibition by the solvent extracts against the Gram positive and Gram negative test organisms.

Organism		Methanol						Ethanol						Acetone					
		Gram positive			Gram negative			Gram positive			Gram negative			Gram positive			Gram negative		
		1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Hot continuous (Soxhlet) extraction	*B. maderaspatensis* leaf	-	-	-	-	-	-	+	++	+	+	+	+	-	+	-	-	-	-
	S. nigrum leaf	+	++	++	-	+	+	+	++	+	+	+	+	+	++	-	-	-	+
	A. indica Leaf	++	+	+	+	+	++	+++	+++	+++	++	++	+	++	+	++	++	+	+++
	A. indica root	+	+	++	+	-	-	+	+	++	+	-	-	+	+	++	+	-	-
	T. procumbens leaf	++	++	-	+	+	++	+	++	-	+	+	+	+	++	-	++	+	+
Cold extraction (Maceration)	*B. maderaspatensis* leaf	++	+	+	+	++	+	+	+	+	+	+	+	+	++	+	+	++	++
	S. nigrum leaf	-	+	-	+	+	++	+	+	-	+	+	+	++	++	-	++	++	++
	S. nigrum stem	-	+	-	-	+	+	-	+	-	++	+++	+	-	-	-	-	-	-
	A. indica leaf	+	+	-	++	++	+	++	++	-	++	++	++	++	+++	-	++	+	+
	A. indica stem	+	++	-	++	+	+	+	++	-	+	+	+	+	++	-	+	+	+
	T. procumbens leaf	-	-	++	-	-	++	-	++	++	+	-	+	+	+++	+++	+	-	+
	T. procumbens flower	+	++	+	+	+	+	+	+++	+++	+	-	+	++	+++	+++	+++	+++	++

Organism		Chloroform						Benzene						Petroleum ether					
		Gram positive			Gram negative			Gram positive			Gram negative			Gram positive			Gram negative		
		1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Hot continuous (Soxhlet) extraction	*B. maderaspatensis* leaf	++	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	S. nigrum leaf	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	A. indica Leaf	++	+	++	++	-	++	+	+	-	++	-	-	-	-	-	-	-	-
	A. indica root	+	+	+++	++	-	-	+	+	+++	++	-	++	++	-	+	+++	-	++
	T. procumbens leaf	+	+	-	+	+	+	+	++	-	+	+	+	-	+	-	+	-	-
Cold extraction (Maceration)	*B. maderaspatensis* leaf	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-		-	-
	S. nigrum leaf	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-
	S. nigrum stem	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-		-	-
	A. indica leaf	+	+	-	++	+	+	-	+	-	+	-	+	-	-	-		-	-
	A. indica stem	-	++	-	+	-	-	+	+	-	+	-	-	-	-	-		-	-
	T. procumbens leaf	-	++	-	-	+	+	-	+	-	-	-	+	-	+	-		-	-
	T. procumbens flower	++	-	-	-	-	-	+	+	-	+	-	+	++	++	-		-	-

1: *Staphylococcus aureus,* 2: *Streptococcus mutants,* 3: *Lactobacillus acidophilus*, 4: *Shigella sonnei*, 5: *Salmonella paratyphi*, 6: *Klebsiella pneumonia*. (-) No zone of inhibition, (+) Zone of inhibition between 11 to 15 mm, (++) Zone of inhibition between 16 to 20 mm, (+++) Zone of inhibition above 20 mm.

bacteria to a greater extent than the Gram negative bacteria. This may be due to the hydrophobic drug principle found in the root parts of *A. indica* that is efficiently extracted by the non-polar solvent benzene extract. Both hot and cold benzene extracts of *A. indica* leaf, *T. procumbens* leaf and flower exhibit some feeble antimicrobial activity. On the other hand, polar solvent extracts of *T. procumbens* leaf, flower and *A. indica* leaf

Table 2. Antimicrobial activity of the reference antibiotics against the Gram positive and Gram negative test organisms.

Organism	Antibiotics used	
	Imipenem	Chloramphenicol
S. aureus	+++	++
S. mutants	++	++
L. acidophilus	+++	+++
S. sonnei	+++	++
S. paratyphi	+++	+++
K. pneumonia	+++	++

(-) No zone of inhibition, (+) Zone of inhibition between 11 to 15 mm, (++)-Zone of inhibition between 16 to 20 mm, (+++)-Zone of inhibition above 20 mm.

had increased the zone of inhibition than non-polar solvent extracts. This gives an inference that some hydrophilic drug principles present in the above aerial plant parts are efficiently extracted by polar solvents, namely, methanol and ethanol than the non-polar solvent benzene.

Comparing the chloroform extracts, both hot and cold extracts of *A. indica* leaf and root exhibited good antimicrobial activity, but comparatively lesser activity than the extracts of polar solvents (ethanol and methanol) and non-polar solvents (petroleum ether and benzene). This gives an indication that the drug principles in the *A. indica* leaf and stem are hydrophilic in nature and they are eluted well using the polar solvents. This also shows that in successive extraction, the drug principles which are hydrophobic in nature are well extracted in the initial non-polar solvents petroleum ether and benzene. On the other hand, hydrophilic drug principle found in the plant parts are later eluted well upon with the successive polar solvent extracts, namely, methanol, ethanol and acetone. Hence, chloroform is a least preferred solvent than the extreme polar and non-polar solvents.

Every solvent in successive extraction will almost elute all the drug principle exhaustively of their respective polarity (Extraction Technologies for Medicinal and Aromatic Plants, 2008); this is in correlation with our finding that the antimicrobial activity exhibited by each solvent extract in our results indicates the activity of drug principle from the plant part that is soluble in that particular solvent system. The antimicrobial pattern of each solvent extract and their phytochemical constituent's analysis of the different solvent extracts consequently obtained in different stages of hot extraction revealed the compounds and elution strength of the respective solvents. The spectrum of compounds varies considerably depending on the hydrophilic or hydrophobic nature of the drug principle.

Analysis of the best solvent system for extracting the drug molecule from each plant part was done by analyzing the antimicrobial activity of all the solvent extracts of the plant parts.

The antimicrobial activity exhibited by the *A. indica* leaf polar solvent extracts is comparatively more than the non-polar solvent extracts. Hence, the ingredients are extracted well by the polar solvents. This finding is in correlation with our earlier finding that acetone and methanol extracts are efficient in extracting the drug principle that control fungal pathogens *Candida albicans* and *Aspergillus flavus* from *A. indica* leaf (Jebakumar Solomon et al., 2005). On the contrary, from *A. indica* root, the non-polar solvents, namely, petroleum ether, benzene and chloroform are efficient in isolating the active ingredients. The polar solvents ethanol and acetone are more efficient in isolating the drug principle from the flower and the leaf.

To our knowledge there is no previous report on the antimicrobial activity and phytochemical analysis of the folkloric medicinal plant *B. maderaspatensis.* In the folkloric medicinal system, there is a practice of using this whole plant extract in water base by just squeezing this plant leaves over the wounds and the microbial borne skin infections. From our experimental results, it is found that both the extreme polar (methanol) and the non-polar solvents (petroleum ether and benzene) are not good in extracting the drug principles, whereas the intermediate solvent extracts like chloroform, acetone, and ethanol are good in extracting the drug principle as observed from their antimicrobial activity.

Polar solvent extracts of *S. nigrum* are exhibiting good antimicrobial activity, whereas there is no report of activity in the non-polar solvent extracts. Hence, polar or the aqueous extracts may be the best solvent system in extracting drug principle from *S. nigrum*. Our findings are in correlation with the reports that *S. nigrum* leaf aqueous extracts are good in controlling the internal ulcers in the enteric tract (Mallika and Chennam, 2006).

The qualitative phytochemical constituent's analysis is shown in Table 3. Further analysis from the antimicrobial

Table 3. Qualitative analysis of the phytochemicals in all the solvent extracts of the best plant part.

	B. maderaspatensis leaf cold						*S. nigrum* leaf hot						*S. nigrum* leaf cold						*A. indica* leaf hot					
	M	E	A	C	B	P	M	E	A	C	B	P	M	E	A	C	B	P	M	E	A	C	B	P
Phenols	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	-	-	-
Sterols	-	-	-	-	-	-	+	+	+	+	-	-	+	+	-	-	-	-	+	+	+	-	-	-
Phlobatannins	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Proteins	+	-	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-	-	+	-	+	+	+
Resins	+	+	+	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-	-	+	+	+	+
Steroid	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	-	-	-	-	-	+	+	+
Tannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+
Acidic compounds	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Terpenoids	+	+	+	-	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	+	+
Reducing sugars	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-

	A. indica leaf cold						*T. procumbens* leaf cold						*T. procumbens* flower cold					
	M	E	A	C	B	P	M	E	A	C	B	P	M	E	A	C	B	P
Phenols	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	-	+	+
Sterols	+	-	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+
Phlobatannins	-	-	+	-	-	-	-	+	+	-	-	-	+	+	-	-	-	-
Proteins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resins	-	-	-	+	+	+	-	-	+	-	+	+	+	-	-	-	-	-
Steroid	-	-	-	+	+	+	-	-	+	-	-	-	+	+	+	+	+	+
Tannins	-	-	-	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-
Glycosides	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acidic compounds	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	+
Reducing sugars	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-

M: Methanol, E: Ethanol, A: Acetone, C: Chloroform, B: Benzene, P: Petroleum ether. (+)-Indicates the presence of phytochemical, (-)- Indicates the absence of phytochemical.

results is projected in Tables 1 and 2. We interpret here the activity of the individual extracts against each test pathogen in comparison with the reference antibiotics imipenem and chloramphenicol. Plant extracts which exhibited antimicrobial activity equivalent or above 20 mm are indicated by (+++) and is considered equivalent to the reference antibiotic. The antimicrobial activity and their responsible phytochemicals in respective solvent activity are discussed subsequently.

The Gram positive pathogens taken in this study are controlled by the polar solvent extracts of *A. indica* and *T. procumbens*. *S. aureus, S. mutant,* and *L. acidophilus are* controlled by *A. indica* leaf, ethanol cold and hot extract, acetone leaf cold and hot extract, *T. procumbens* leaf and flower acetone cold extracts. The responsible phytochemicals in respective solvent extracts are *A. indica* leaf acetone cold extract has phlobatannins, proteins and terpenoids. Ethanol hot extract has phenols, sterols, proteins and

terpenoids. *T. procumbens* leaf acetone cold extract has terpenoids, glycosides, tannins, steroids, proteins and phlobatannins.

The Gram negative pathogens *S. paratyphi, S. sonnei,* and *K. pneumonia* is controlled by *A. indica* leaf acetone hot extract, *S. nigrum* ethanol cold and *T. procumbens* flower acetone cold extract (Ayyappa Das et al., 2009). The responsible phytochemicals in respective solvent extracts are *A. indica* leaf acetone hot extract has phenols, steroids, resins, and terpenoids. *S. nigrum* ethanol cold and *T. procumbens* flower acetone cold extract has phenols, sterols, phlobatannins, proteins, tannins and terpenoids.

Conclusion

It is observed in general from all the plant parts taken in this study that polar solvents are good in extracting the drug principle from the aerial parts of the plant like leaf and flower, while the non-polar solvent benzene and petroleum ether are good in extracting drug principle from the root part of the plant. The mode of extraction and the temperature employed in extraction determines the extract's activity/efficiency; the hot and cold extract of the same solvent exhibits dissimilar activity.

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