Open Access Contents
Int. J. Drug Dev. & Res.
| July-September 2013 | Vol. 5 | Issue 3 | ISSN 0975-9344 |
www.ijddr.in

Comparative analysis of Medicinal Plants for their Antimicrobial Potential and Phytoconstituents Screening

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Abstract: The present study was designed to evaluate the antimicrobial efficacy of crude extract of the leaves and stems of *Amaranthusspinosus L.*, *Capparisdeciduas* (kair), *Chenopodium album* (bathua) and *Salvadorapersica*(meswak/jal)against pathogenic microbial strains *Bacillus subtilis, E.coli, Bacillus amyloliquefaciens, Staphylococcus aureus, Staphylococcus epidermidis*and *Streptococcus mutans*assayed by using agar well diffusion assay. Four different extracts (acetone, benzene,methanol and cow urine) of each plant were used during the study. The significant results were obtained by all solvent extracts except cow urine extracts on tested pathogens using Agar well diffusion method. Acetone extract revealed strongest antibacterial activity on *E.coli*and methanol leaf extract showed strongest antibacterial activity on *Staphylococcus epidermidis.*Preliminarily phytochemical investigation of the crude extract of the leaves and stems of plantsshowed the presence of tannin, alkaloids, glycoside, terpenoids, flavonoid, steriods and saponin. The presence of these secondary metabolites indicates the pharmacological property of the plant leaves and stem. On the basis of this finding, the extracts demonstrating antimicrobial efficacy could result in the discovery of novel antimicrobial agents.

Keywords: Leaves and stems, Solvent Extract, Antibacterial activity, Phytochemical analysis.

Introduction

Since the beginning of human civilization, medicinal plants have beenused by mankind for its therapeutic value. Nature has been a source ofmedicinal agents for thousands of years and an impressive number ofmodern drugs have been isolated from natural sources. According to the World Health Organization WHO [1] amedicinal plantis any plant, which in one or more of its organs containsubstances that can be used for the therapeutic purposes or which areprecursors for the synthesis of useful drugs.

Medicinal plants containactive chemical constituents in any of their parts like root, stem, leaves, bark, fruit and seeds. These compounds either act on different systems ofanimals including man and act through interfering in the metabolism of microbes infecting them. In either way the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favors of the host. The medicinal properties of plants could be based on the antioxidant, antibacterial and antifungal effects of thephytochemicals in them [2].

According to a report of world health organization (WHO)[1], 70% of the world population uses medicinal plants to cure diseases through their traditional practitioners. India has several traditional medical systems, such as Ayurveda and Unani,which has survived through more than 3000 years, mainly using plant-based drugs. In Indian subcontinent, plant oriented drugs have been used extensively from a very long time. According to a survey conducted by WHO, traditional healers treat 65% patients in Sri lanka,

60% in Indonesia, 75% in Nepal, 85% in Myanmar, 60% in Pakistan, and 90% in Bangladesh. In India, 80% of the population especially in villages is getting health care by traditional practitioners (Hakims) who prescribe herbal preparations [3].Modern strategies for drug discovery emphasize on availability of some simple and inexpensive biological assays to evaluate medicinal potential of plant species. So in our present study various plants used by traditional healers were analyzed for their medicinal value.

Salvadorapersica (Jal or Meswak) has been used by many Islamic communities as toothbrushes. Chewing sticks that are made from the roots, twigs or stems of *S. persica*are commonly used in the Middle East as a means of maintaining oral hygiene. Studies indicate that *S. persica* extract when used at the higher concentration is somewhere comparable to other oral disinfectants and anti-plaque agents such as triclosan andchlorhexidinegluconate[4].

The various parts of *Amaranthusspinosus* are known to possess various pharmacological properties[5].Extracts and leaves are used in treatment of menstrual disorders in women[6]. An infusion of powdered seeds of the plant is used for stomach problems and to alleviate labor pain in the pregnant women in Nepal[7].Further it is used to treat several ailments like gonorrhea, inflammatory swelling, malaria, diabetes,leprosy[8][9].

Capparisdeciduas plant is used in treatment of asthma, gout, rheumatism, ulcer, ear infection as well as in some skin disorders[10]. The parts of *Chenopodium album* L. (Bathua) are useful in curing anorexia, cough, dysentery, and diarrhea, piles and kills small worms.

Materials and Methods:

Collection of plant materials: The fresh stems and leaves of *Amaranthusspinosus*, *Capparisdeciduas* (kair*)*, *Chenopodiumalbum* (bathua) and*Salvadorapersica* (meswak/jal*)* were collected from Hisar (Haryana, India) region.

Collection of Microbial cultures:

The pathogenic organisms were procured from the Institute of Microbial Technology (IMTECH); Chandigarh, India. The antimicrobial activity of the extracts was tested individually on bacterial and fungal strains.

Determination of antimicrobial activity:

Procuring of bacterial strain:

Pure cultures of test organisms were procured from IMTECH, Chandigarh, India (Table1).

Table 1: List of microorganisms used in the study:

Bacterial Strain	MTCC NO.
Bacillus subtilis	121
E.coli	483
Bacillus amyloliquefaciens	1488
Staphylococcus aureus	96
Staphylococcus epidermidis	435
Streptococcus mutans	890

Preparation of Standard Culture inoculums

A loop full of the different strains were inoculated in 25 ml nutrient broth in a conical flask and incubated at room temperature on a rotary shaker for 24 hr. The optical density was measured at 625 nm after 24 hrs using a spectrophotometer. The prepared culture was appropriately diluted with nutrient broth to achieve sufficient inoculum. The Bacterial strains were maintained on nutrient agar at 4 ◦ C and sub cultured every month.

Determination of antimicrobial activity by agar well diffusion method [11]:

The leaves and stems of plants under study were air dried, powdered and 50 g of each was macerated with organic solvents viz. acetone, Benzene, Methanol, cow urine (Sigma Aldrich ltd) for 3-7 days at room temperature. Filtration of soaked material was done by using whatman filter paper. The aqueous part of the crude plant extract was dried by using a freeze-drier.

Petri plates containing 20ml of Nutrient agar (Himedia, Mumbai) were inoculated with 100µl of diluted bacterial by the spread plate technique and were allowed to dry in a sterile chamber. A well of about 7.0 mm was aseptically punctured with sterile cock borer. A 150µl of the extract (200mg/ml) were loaded into the wells and were allowed to dry completely. Methanol was used as a negative control whereas cefotaxime was used as positive control. Plates were incubated at 37°c for 24hrs. The antibacterial activity was assessed by measuring the zone of inhibition.

Phytochemical Analysis of Extract

The solvent extracts of different plants were subjected to preliminary phytochemical screening to identify the chemical constituents[12].

Results and discussion

Medicinal herbs as potential source of therapeutics aids has attained a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaining proper health. Results for antibacterial activity and antifungal activity illustrated that leaf and stem extract of medicinal plants have significant high activity against all the bacterial and fungal strains tested but cow urine used for plant extract preparation did not give any activity against any of bacterial strains (Table 2). Sothese extract can be fractionated in future to get active components responsible forantibacterial activity.

Table 2: Antibacterial activity of extracts of medicinal plants against bacterial strains.

Plants	Solvent Extract	*B.amyloliquefaciens*	*B.subtilis*	*E.coli*	*S.aureus*	*S.epidermidis*
		Diameter of zone of inhibition in millimeters				
A. spinosusL.	Acetone	_	9	12.5	14.5	12.5
	Benzene	_	12	_	_	_
	Cow Urine	_	_	_	_	_
	Methanol	_	13	16	14	13
C.decidua	Acetone	_	_	24	17.5	14
	Benzene	_	15	_	_	10
	Cow Urine	_	_	_	_	_
	Methanol	_	_	17	15	18
C. album	Acetone	10.5	_	_	_	_
	Benzene	_	12.5	_	_	_
	Cow Urine	_	_	_	_	_
	Methanol	_	17.5	15.5	15	7
S. persica stems	Acetone	11.5	_	21	_	12.5
	Benzene	_	16.5	_	_	_
	Cow Urine	_	_	_	_	_
	Methanol	12	15	15	_	15
S. persica leaves	Acetone	9	_	_	_	17
	Benzene	11.5	_	_	_	_
	Cow Urine	_	_	_	_	_
	Methanol	_	9.5	12	_	10

Our two of the four solvent extracts (Acetone and Methanol extracts) presented significant antimicrobial activity against almost all the strains through agar well diffusion technique. The results revealed that *E.coli, S.aureus and S.epidermidis*were found to be highly sensitive against *C.decidua* and *S.persica* extracts (Fig. 1).

The methanol extracts of six plant species including *Salvadorapersica* exhibited antimicrobial activity against pathogens namely *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*by disc diffusion method[13].

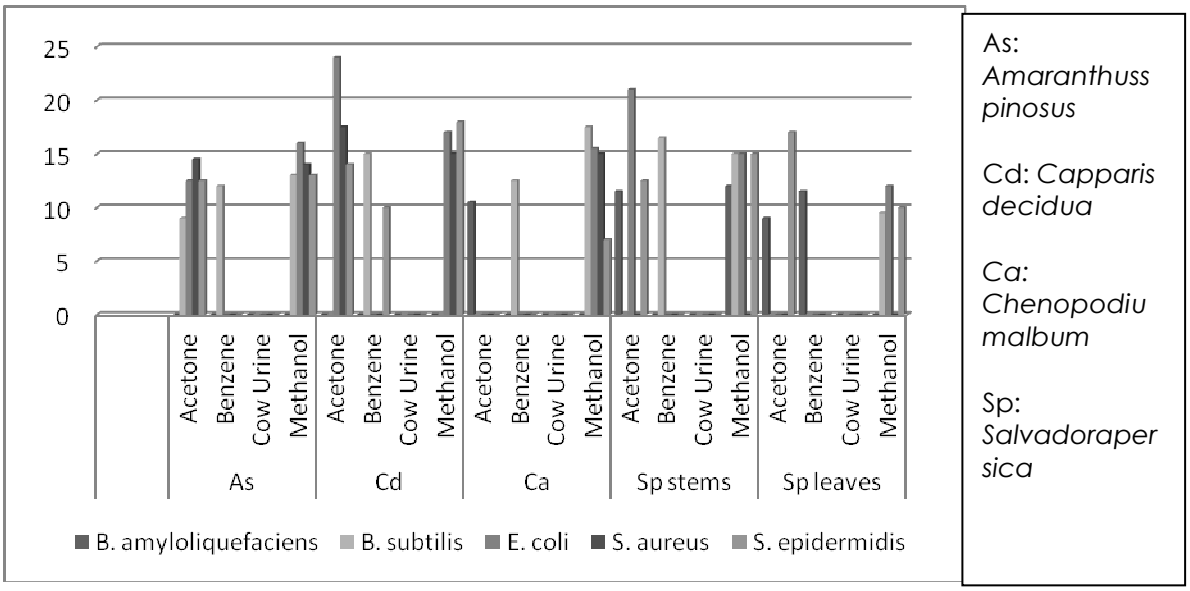

Fig 1: Antibacterial activity of extracts of medicinal plants against bacterial strains

In the present study, the methanol and acetone leaf extracts of *Amaranthusspinosus L.* exhibited maximum antibacterial activity against*E.coli and Staphylococcus aureus*with 16 mm and 14.5 mmzone of inhibition respectivelywhilemethanol extract of *C.album* was found to be highly effective against *B. subtilis* with 17.5 mm zone of inhibition. These observations were in conformity with the [14]who analyzed antimicrobial activity of aqueous and methanol extracts of *Chenopodium* leaves against human pathogenic bacteria viz. *Escherichia coli, Salmonellatyphimurium, Staphylococcus aureus, Proteus vulgaris* and *Pseudomonas aueruginosa*and found thataqueous extract revealed strongest antibacterial activity on *Staphylococcus aureus.*

Phytochemical screening of the leaf and stem extracts of plants under study revealed the presence of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, saponins, phenol and reducing sugars (Table 3). These compounds have significant application against human pathogens, including those that cause enteric infections and are reported to have curative properties against several pathogens and therefore could suggest their use in the treatment of various diseases[15]. Methanol extract of all plants showed the presence of flavonoids and tannins whilesaponins and phenols were absent.This is in confirmation with[16]who reported better phytochemical extraction in methanol. Tannins have been found to form irreversible complexes with prolinerich protein[17] resulting in

the inhibition of cell protein synthesis. Tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues[18]. Herbs thathave tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery[19].

Another secondary metabolite compound observed in the stem and leaf extractsof plantswas alkaloid. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines[20]. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications [21].

Apart from tannin and alkaloids compounds, other secondary metabolite constituents of all the plants included flavonoids, glycosides, carbohydrates, steroids and aldehyde. Flavonoids are potent water-soluble, antioxidants and free radical scavengers which prevent oxidative cell damage[22]. Acetone and benzene extracts showed presence of only few phytochemicals while cow urine extracts failed to show presence of any phytochemical constituent. However antimicrobial activity and presence of phytochemical constituents in cow urine extracts have been reported by many researchers [23][24].

It is not surprising that there were differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. It is quite possible that some of theplants that were ineffective in this study do not possess antibiotic properties, or the plant, extracts may have contained antibacterial constituents, just not in sufficient concentrations so as to be effective. It is also possible that the active chemical constituents were not soluble in used solvents.

Table 3: Preliminary phytochemical analysis of medicinal plants

Phytochemical Constituents	Acetone					Benzene					Cow Urine					Methanol					
	A.spinosusL.	*C. deciduas*	*C.album*	*S. persica stems*	*S. persica leaves*	*A.spinosusL*	*C.deciduas*	*C. album*	*S. persica stems*	*S. persica leaves*	*A.spinosusL.*	*C. deciduas*	*C.album*	*S. persica stems*	*S. persica leaves*	*A.spinosusL.*	*C. deciduas*	*C.album*	*S. persica stems*	*S. persica leaves*	
Aldehyde	Tollen's test	–	+	+	+	–	–	+	–	+	+	–	–	–	–	–	+	+	–	+	+
Alkaloids	Hager's test	+	+	–	–	+	–	+	–	+	+	–	–	–	–	–	+	+	+	+	+
Carbohydrate	Molisch's test	+	–	–	+	+	+	+	+	+	+	–	–	–	–	–	–	+	+	+	+
Flavanoids	NaOH test	+	–	+	+	+	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+
Glycosides	Keller Test	–	+	+	+	+	–	+	+	–	–	–	–	–	–	–	+	+	+	+	–
Phenols	$FeCl_3$ Test	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
Steroids		–	–	–	+	+	–	+	+	+	–	–	–	–	–	–	+	+	+	–	+
Saponins	Foam Test	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
Terpenoids		–	–	+	+	–	–	–	+	–	–	–	–	–	–	–	+	–	+	+	+
Tannins	Fecl3	–	–	–	+	+	–	–	–	–	+	–	–	–	–	–	–	+	+	+	+

The drying process may have caused conformational changes to occur in some of the chemical constituents found in these plants. The environmental, climatic and geographical distributions can also play a major role.

Conclusion

Antibiotics resistance is increasing day by day among micro-organisms due to the spread of resistant genes via plasmid throughout other species, eventually limiting the efficacy of various drugs. The priority for the next generation or decades must be focused in the development of alternative drugs and/ the recovery of molecules that would allow the consistent and proper control of microorganisms which cause diseases. Ideally, these molecule should be as natural as possible with a wide range of activity over several harmful bacteria and fungal strains, easy to produce and not prone to induce resistance, with minimal residual effect.

The present study reveals the scientific validation of natural medicinal plant products for the use as antimicrobial agents. The presence of wide range of phytochemicals present in the plants may be a reason for the antimicrobial activity possessed by the plants.The demonstration of broad spectrum activity of medicinal plantsmay help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. The effect of these plants on more pathogenic organisms, and toxicological investigations and further purification, however, need to be carried out

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Covered in Scopus & Embase, Elsevier **Int. J. Drug Dev. & Res., July-September 2013, 5 (3): 447-453**

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Article History:----------

Date of Submission: 06-06-2013

Date of Acceptance: 29-07-2013

Conflict of Interest: NIL

Source of Support: NONE