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ANTIBACTERIAL EVALUATION OF SOME COMMON MEDICINAL PLANTS USED IN ASAVA AND ARISHTA PREPARATION

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ABSTRACT: Various medicinal properties have been attributed to natural herbs. The use of traditional medicine is widespread in India. These herbal remedies are an important source for the discovery of new antimicrobials against the resistant strains of bacteria. Asavas and Arishtas are specially prepared ayurvedic medicines. They are alcoholic solutions containing all the active ingredients of the drugs of which they are compounded. Asavas are prepared by using herbal juices or herbs soaked in water while Arishtas are prepared by decoction. Different parts of medicinal plants are used traditionally for the preparation of specific asava or arishta that is effective against specific disorder. About 25 different medicinal plants commonly used were selected. Dried powders of these plants were extracted in water. The antibacterial activity of the extracts were then tested by agar well diffusion method against standard cultures of *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Bacillus thuringenesis.* The individual extracts of *Emblica officinalis, Terminalia chebula, Terminalia belerica* and *Woodfordia fructicosa,* Jaggery were active against the test pathogens. A combination of these raw materials as cited in literature commonly called as Lohasava, Punnarnavasava, Amritarishtha and Kirayatikadha were prepared and were found to be more effective than individual raw materials.

INTRODUCTION: Nature has been the source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be overemphasized [1]. World Health Organization indicates that primary health needs of countries in Africa, Asia and Latin America are met by traditional medicines. Such traditional medicines are adapted to industrialized countries as Complementary or Alternative Medicines (CAM) [2].

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Ayurvedic system of treatment has been estimated to meet 70 – 80% of healthcare needs of India [3].

Asavas and arishtas are self-generated herbal fermentations of traditional Ayurvedic system. They are alcoholic medicaments prepared by allowing the herbal juices or their decoctions to undergo fermentation with the addition of sugars. Arishtas are made with decoctions of herbs in boiling water while asavas are prepared by directly using fresh juices [4-8].

Fermentation of both preparations is brought about by the addition of a source of sugar with dhataki (*Woodfordia fructicosa* Kurz) flowers [4]. They are moderately alcoholic and mostly sweetish with slight acidity and agreeable aroma.

These medicinal wines have several advantages, like better keeping quality, enhanced therapeutic properties, improvement in the efficiency of extraction of drug molecules from the drugs and improvement in drug delivery into the human body sites [9].

It is clear that plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases [1]. More than 1200 species of plants, nearly 100 minerals and over 100 animal products comprise the Ayurvedic Pharmacopoeia [10]. The active components of the herbal medicines have the advantage of being combined with many other substances that appear to be inactive. However these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [1].

Antibiotic resistance has become a global concern [11]. There has been an increasing incidence of multiple resistances in human pathogenic micro-organisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced the scientist to search for new antimicrobial substances from various sources like medicinal plants [12]. These medicinal plants constitute the main source of new pharmaceuticals and healthcare products [13].

Extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity profile drugs [14]. Thus, the need of the hour is to screen a number of medicinal plants for promising biological activity. In the present work, 25 different medicinal plants commonly used for the asava and arishta preparation were evaluated for their antibacterial properties. Also, a combination of these raw materials was prepared as reported in literature and its antibacterial activity was also evaluated.

MATERIALS AND METHODS:

Plant material: Different parts of the plant material commonly used were collected from the local vendor. The details of the part used, their families are given in **Table 1.** The dried plant parts were then homogenized to fine powder and then stored in airtight bottles.

TABLE 1: PLANT PARTS SCREENED FOR ANTIBACTERIAL STUDY

Botanical Name	Family	Local Name	Plant Parts used
Swertia chirata	Gentianaceae	Chirayata	Plant
Zingiber officinale	Zingiberaceae	Sunthi	Rhizome
Saccharum officinarum	Poaceae	Gud	Root stock
Woodfordia fructicosa	Lythraceae	Dhayatiphool	Flower
Cuminum cyminum	Apiaceae	Jira	Fruit
Piper nigrum	Piperaceae	Mire	Fruit
Piper longum	Piperaceae	Pippali	Fruit
Mesua ferrea	Calophyllaceae	Nagkeshar	Stamens
Picrorhiza kurroa	Scrophulariaceae	Kutki	Root
Holarrhena antidysentrica	Apocynaceae	Indrajav	Seed
Bunium persicum	Apiaceae	Kalejire	Fruit
Terminalia chebula	Combretaceae	Harada (Haritaki)	Pericarp
Terminalia belerica	Combretaceae	Behada (Bibhitaki)	Pericarp
Emblica officinalis	Phyllanthaceae	Amla	Pericarp
Trachyspermum ammi	Apiaceae	Ova	Fruit
Ricinus cemmunis	Euphorbiaceae	Aerandmul	Root
Boerhaqvia diffusa	Nyctaginaceae	Punnarnava	Root
Azadirachta indica	Meliaceae	Nimchaal	Stem bark
Trichosanthes cucumerina	Cucurbitaceae	Kadupadwal	Fruit
Fagonia *arabica*	Zygophyllaceae	Dhamasa	Leaves
Cyperus rotundus	Cyperaceae	Nagarmotha	Rhizome
Lohachurna	--	--	--
Berberis aristata	Berberidaceae	Daaruharad	Root
Tribulus terustris	Zygophyllaceae	Gokharu	Root
Solanum xanthocarpum	Solanaceae	Kantkari	Fruit

Preparation of the Extract: For aqueous extract preparation, 10g of dried powder was added to distilled water and boiled on slow heat for 2hrs. It was then filtered through eight layers of muslin cloth and centrifuged at 5000g for 10 mins. The supernatant was collected. This procedure was repeated twice. After 6 hrs, the supernatant collected at an interval of every 2hrs was pooled together and concentrated to make a final volume one-fourth of the original volume [14]. It was then autoclaved at 121^{o}C temperature i.e. 15lbs pressure and stored at 4^{o}C.

As reported in the literature, some combinations of these raw materials were made. Two asavas and two arishtas were prepared. Again a new combination of these asavas and arishtas was done and a novel antibacterial agent was prepared.

The first combination among the asavas is reported as lohasava and the second one as punnarnavasava. The ingredients for these two preparations are as follows;

1. Lohasava – lohachurna, sunthi, mire, pippali, harada, behada, amla, vavading, nagarmotha, chitraka, aerandmul, dhayatiphool, gud and honey.

2. Punnarnavasava – punnarnava, sunthi, mire, pippali, harada, behada, amla, dashmul, gokharu, kanthakari, adudasa, kutki, gajpippali, nimchaal, kadupadwal, shinganimul, dorlimul, aerandmul, dhamasa, gudwel, sugar, gud and dhayatiphool.

Among the arishtas, the first reported is amritarishta and the second one as kirayatikadha. The ingredients for these both are as follows –

1. Amritarishta – dashmul, gudwel, jira, sunthi, mire, pippali, nagkeshar, nagarmotha, kutki, sugandhbala, indrajav, kalejire, dhayatiphool, gud.

2. Kirayatikadha – chirayata, nagarmotha, gudwel, sugandhbala, usher, sunthi, gud and dhyatiphool.

Bacterial inoculum preparation: *In-vitro* antimicrobial activity was examined for different plant extracts from medicinal plants commonly used for the asava and arishta preparation by traditional healers. The micro-organisms were sub cultured from the stock cultures maintained in the microbiology laboratory of our college. The micro-organisms were maintained at 4^{o}C on nutrient agar slants. Amongst the micro-organisms investigated two Gram positive bacteria were *Staphylococcus aureus* and *Bacillus thuringenesis* while four Gram negative bacteria were *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Salmonella typhimurium*.

Antimicrobial assay: The antimicrobial assay was performed by agar well diffusion method [16]. The molten Mueller - Hinton agar (Himedia) was inoculated with 100µl of the inoculum and poured into the sterile petri-plates. For antimicrobial assay, a well was prepared in the plates with the help of a cork-borer (0.7cm). 100µl of the test compound was introduced into the well. The plates were incubated overnight at 37^{o}C.

Microbial growth was determined by measuring the diameter of the zone of inhibition. The experiment was done three times and the mean values are presented. The results were compared with the standard antibiotic discs Penicillin-G, Bacitracin, Ampicillin and Chloramphenicol (10µg/disc).

RESULTS AND DISCUSSION: Medicinal plants constitute an important source of both traditional and modern medicines. Herbal medicines have been shown to have genuine utility and about 80% of rural population depends on it as primary healthcare. Over the years, the World Health Organization (WHO) advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins.

The present study was conducted to investigate antibacterial properties of some common medicinal plants used for the preparation of some asavas and arishtas. Different parts of total 25 medicinal plants were extracted with water and their antibacterial activities were studied. As cited in literature, four combinations of these raw materials was prepared and the antibacterial activity was studied **(Table 2)**. Amongst the selected plants for study, fruits of *Emblica officinalis, Terminalia chebula, Terminalia belerica* proved to be the strongest antibacterial followed by the flowers of *Woodfordia fructicosa*,

Saccharum officinarum and so on. *Bacillus thuringenesis* was the most sensitive and *Salmonella typhimurium* was the most resistant test pathogen.

Salmonella typhimurium and *Klebsiella aerogenes* were the most resistant bacteria. Only the individual extracts of *Emblica officinalis, Terminalia chebula* and *Woodfordia fructicosa* could inhibit *S. typhimurium. Kl. aerogenes* was inhibited by only *Emblica officinalis, Terminalia chebula* and *Terminalia belerica.* On the other hand, a Gram positive bacterium, *B. thuringenesis* was the most susceptible followed by *S. aureus*. Various workers have already shown that Gram positive bacteria are more susceptible towards plant extracts as compared to Gram negative bacteria [17-18]. These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of single layer, whereas Gram negative cell wall is multilayered structure [19]. It is thought that observed differences may result from doses used in the study. In addition, micro-organisms show variable sensitivity to chemical substances related to different resistant levels between strains [20].

The three raw materials namely *Emblica officinalis, Terminalia chebula* and *Terminalia belerica* are components of the wonderful Ayurvedic drug – Triphala that acts as a perfect tonic for proper digestion [21]. Triphala has many specific effects. It is particularly rejuvenating for the digestive tract, and also cures dyspepsia, anaemia, impurity of blood, hyperlipidaemia, skin diseases, excessive heat and irritation of eyes [22]. The activity of the individual raw materials supports these findings. Antibacterial activity of the flowers of *Woodfordia fructicosa* was recorded [23]. The results were parallel to our findings. Sensitivity against *S. aureus* supported flowers use as an antiseptic agent.

TABLE 2: ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS AND THEIR COMBINATIONS (DIAMETER OF ZONE OF INHIBITION MEASURED IN mm)

Botanical names	*E. coli*	*S. aureus*	*P. vulgaris*	*S. typhimurium*	*B. thuringenesis*	*Kl. aerogenes*
Swertia chirata	--	15	--	--	12	--
Cyperus rotundus	--	--	--	--	12	--
Zingiber officinale	--	--	11	--	13	--
Saccharum officinarum	19	24	13	--	--	--
Woodfordia fructicosa	10	16	18	12	18	--
Cuminum cyminum	--	--	14	--	13	--
Piper nigrum	14	--	--	--	--	--
Piper longum	--	--	13	--	10	--
Mesua ferrea	--	--	--	--	11	--
Picrorhiza kurro	--	--	10	--	13	--
Holarrhena antidysentrica	14	16	<10	--	--	--
Cuminum cyminum (black)	-	12	--	--	18	--
Lohachurna	--	--	--	--	--	--
Terminalia chebula	22	26	23	15	22	14
Terminalia belerica	35	24	22	--	21	12
Emblica officinalis	20	28	25	23	24	26
Trachyspermum ammi	--	13	--	--	15	--
Ricinus cemmunis	--	15	12	--	--	--
Boerhaqvia diffusa	--	12	14	--	--	--
Berberis aristata	--	18	--	--	--	--
Tribulus terustris	--	10	--	--	--	--
Solanum xanthocarpum	--	--	--	--	11	--
Azadirachta indica	--	13	--	--	10	--
Trichosanthes cucumerina	--	10	--	--	--	--
Fagonia Arabica	42	25	--	--	--	--
Lohasava	26	28	14	23	15	12
Punnarnavasava	--	19	15	13	11	15
Kirayatikadha	--	13	13	11	--	14
Amritarishta	--	13	13	11	12	12

Some of the investigated plants do not show any/strong antibacterial activity. However, negative results do not mean absence of bioactive constituents nor is that plant inactive. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with dose levels employed [24]. Lack of activity can thus only be proven by using large doses [25]. Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents [26]. With no antibacterial activity, extracts may be active against other bacterial species which were not tested [27].

The different combinations of the raw materials which were made gave significant zone of inhibition which probably explains the use of these combinations by Ayurvedic practitioners. Some individual raw materials used did not show any antibacterial activity however the combination gave a significant result. This may be due the synergistic effect during the process of fermentation. This synergistic effect needs to be studied further and some other combinations also need to be tried.

CONCLUSION: Plants are potent biochemists and have been components of phytomedicine since tmes immemorial [28]. From the results, it can be concluded that the aqueous extracts of *Emblica officinalis, Terminalia chebula, Terminalia belerica* and *Woodfordia fructicosa* exhibited significant antimicrobial activity and properties that support its use in the treatment of some diseases. This probably explains the use of these plant parts by traditional healers against a number of infections. The medicinal plants exhibiting remarkable antimicrobial activity can be further subjected to isolation of therapeutic antimicrobials and carry out further pharmacological evaluation.

The study of the phytochemicals from these plants may reveal some new antimicrobials. Again different combinations of these raw materials which were made showed significant antimicrobial activity. The synergistic effect responsible for this activity needs to be studied in detail and some new combinations should also be made.

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