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RESEARCH ARTICLE

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACT OF DIACURE A POLYHERBAL FORMULATION

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ABSTRACT

Diacure a polyherbal formulation of eleven medicinal plants against some Microorganisms was investigated for this purpose methanol extract of diacure samples were in vitro with *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis, Micrococcus luteus, Escherichia coli and Candida albicans as test strains. The disc diffusion method* was applied in the trial was found to be the most effective against all the test strains except *M. luteus*.

INTRODUCTION

According to world health organization (WHO) more than 80% of the world Population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illness may be expected to have accumulated in areas where the use of plants is still of great importance (Diallo *et al.,* 1999). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most importance of these bioactive compounds of plants is alkaloids, flavonoids, tannins and phenolic compounds. The use of medicinal plants as traditional medicines is well known in rural *areas of many developing countries* (Sandhu *et al., 2005;* Gupta *et al.,* 2005).

Traditional healers claim that their medicine is cheaper and more effective than modern medicine. In developing countries low income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections (Rojas *et al.,* 2006). We chose Diacure, a polyherbal formulation of 11 medicinal plants to determine the antimicrobial activity (Table 1).

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In general these plants are used in folk medicine in the treatment of skin diseases, venereal diseases, and respiratory problems. Evidently there are many scientific studies that confirm the antimicrobial properties for most of the plants collected for this study (Kloucek P).

MATERIAL AND METHODS

Plant materials

Ethno Botanicals Survey: Plants were selected for this study based on their medicinal use. Fresh plant parts were collected from the tribal villages in Palani Hills of Tamilnadu, India. The voucher specimens in duplicate were deposited in herbarium Department of Botany, Annamalai University (India). Here we are using Diacure a polyherbal formulation of 11 medicinal plants (Table 1).

Preparation of the Methanol extract: For the preparation of the extract the method reported by Hanafy and Hatem (19 91) was used. For this purpose, 50 ml of methanol was added into 20 g of powdered plant materials and the mixture was left for 6 hours. The mixture was periodically agitated during this period (15 min). Afterwards, it was filtered and the methanol was vaporized in an evaporator (60−C). The dark colored oily extract obtained at the end of these processes was used in a non diluted form for the analysis. Antimicrobial activity tests were started on the same day. The sample extracts were kept in the refrigerator (4−C) until the analysis was Accomplished.

Test microorganism: The following microorganisms which cause food poisoning or food spoilage and a yeast culture were used as test strains viz, *Staphylococcus aureus, Klebsiella pneumoniae, Pseudo monas aeruginosa, Enterococcus faecalis, Micrococcus luteus, Escherichia coli and Candida albicans* provided by easma institute of technology, karur, tamilnadu, India.

***Bacterial isolation and pure culture*:** There are different types of media were used for bacterial isolation. Like Zobell's Marine Agar 2216, Mac Conkey's Agar (MAC), Vibrio agar, Nutrient Agar, Nutrient Broth were used for the isolation and identification of some Gram- Positive and Gram - Negative bacteria.

Indole test: Tryptone agar was inoculated and incubated at 37 °C for 48 hours and added Kovacs reagent and read immediately. They results were interpreted based on the change of color from yellow to pink.

Methyl red test (MR): Buffered glucose broth was inoculated and incubated at 37o C for 48 hours. A few drop o f methyl red solutions were added to culture and the results were read immediately. The results were interpreted based on the change of color from yellow to red .

Voges Proskauer test (VP): The organisms were inoculated in buffered glucose broth and were incubated at 37 °C for three days and 3 ml of alpha napthol was added followed by 1 ml o f 40% KOH. It was mixed well and allowed to stand for 30 min. The results were interpreted based on the change of yellow color to pink.

Table 1. Diacure, a polyherbal formulation of 11 medicinal plants to determine the antimicrobial activity

S.No	Name of the plant	Common name	Family	Part of the plant used
1	*Syzigium cumini*	Jamun	Myrtaceae	Seed
2	*Azardiricta indica*	Neem	Meliaceae	leaves
3	*Ocimum tenuiflorum*	Tulsi	Lamiaceae	leaves
4	*Abitulon indicum*	Tuthi	Malvaceae	Leaves
5	*Cassia auriculata*	Cassia	Ceasalpinaceae	Flower
6	*Ficus bengalensis*	Aalam	Moraceae	Seed
7	*Tinospora cardifolia*	Seendal	Convolvulaceae	Root
8	*Phyllanthus emblica*	Nelli	Euphorbiaceae	Seed
9	*Trigonella foenagraceum*	Fenugreek	Leguminasae	Seed
10	*Curcuma longa*	Turmeric	Zingiberaceae	Rhizome
11	*Phyllanthus niruri*	Keelanelli	Euphorbiaceae	leaves

The Gram Stain: A heat fixed bacteria smear was covered completely with a few drops of crystal violet solution. After 30- 60 sec the smear was rinsed with water by squirting the slide above the smear and letting the water wash over it until the water runs clear. Several d rops of iodine (mordant) was app lied to cover the smear and left for 60 sec then rinsed again. A few drops of Isopropanol-acetone mixture
was added at a time until it become colorless, then the slide was rinsed again. Aqueous safranin was applied for 30- 60 sec follo wed by a rinse. The smear was blotted to remove excess water, using absorbent paper. The slide was then air dried and observed under a microscope.

Motility: For Motility Test Medium was used to check the motility of the bacterium. Bacterial motility can be shown using differen t types of motility medium. The composition of these preparations gives freedom of movement co mparable to that of broth culture Motile bacteria were identified by the presence of growth away from the line of inoculation whereas non – motile organisms grow only the initial stab line.

Biochemical tests

Catalase test: This test was conducted to detect the presence of the enzyme Catalase. A capillary tube was dipped into 3% H_2O_2 and the colony was touched.

Citrate utilization: The organisms were streaked onto Simmons Citrate agar plate and incubated at 37 °C for 24hours. The results were interpreted based on the change of color from initial green to deep blue if it was positive.

Antimicrobial tests: The disc diffusion method was used to determine the antimicrobial activity of the spices. A volume of 0.1 ml of the tested microorganisms grown in liquid growth media (at 37°C for 24 hrs, 108-109 cells/ml), was inoculated on Mueller- Hinton growth media, and then spread on the entire surface of the dish using a sterile spatula. Then, sterile paper discs (Whatman: 1.6 mm) with absorbed diacure methanolic extract (80µl, 90 µl, 100 µl/ disc) were placed onto the agar at certain intervals by pressing gently. After the plates were incubated at 35 °C for 48 hours, the inhibition zones around the discs where no growth occurred were measured in millimeters. The experiments were repeated in duplicate for all of the test strains.

Antifungal activity: Petri dishes with potato dextrose agar were inoculated with three different Millipore's filter (Millipore, USA) round disc with a radius of 1.6 mm were dipped in to each sample extracts and placed in the centre on inoculated petridishes. Fungus colonies were allowed to grow 48 hours at 28 °C and then the inhibition zone around the disc was measured.

RESULTS AND DISCUSSION

Table 1 provides the botanical name, family, plant parts used together mixed in 1:1 ratio forming a polyherbal formulation of 11 medicinal plants. Methanolic extract of diacure showed antimicrobial activity by inhibiting one or more

microorganisms. The results of the antimicrobial screening of the methanolic extract were shown in Figure 1 below. The tested plant extracts were most active against gram positive microorganisms than the gram negative microorganisms. This is in agreement with the previous reports by several workers (Gupta *et al.,* 2005).

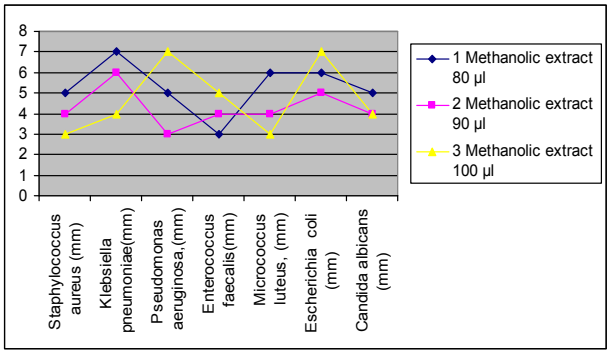

Fig. 1. The antimicrobial activity of diacure a polyherbal formulation

as Syzigium cumini showed good activity against many microbes (Kloucek *et al.,* 2005**)** as reported by Rajakaruna etal also. In previous findings flower, roots, and stem of some medicinal plants showed a range of activity against several bacteria and protozoa. (Diallo *et al.,* 1999). In this study methanolic extract of diacure a polyherbal formulation of 11 medicinal plants showed antibacterial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis, Micrococcus luteus, Escherichia coli and Candida albicans, of* concentrations 80, 90 and 100µl/disc. Of which

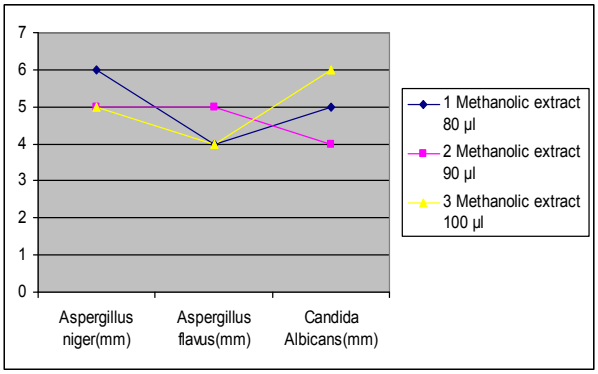

Fig. 2. The antifungal activity of diacure a polyherbal formulation

methanolic extract of 100µl/disc concentration showed greatest activity against *Pseudomonas aeruginosa, Escherichia coli* as compared with other organisms tested. Then 80µl/disc concentration showed greatest activity against *Klebsiella pneumoniae, Micrococcus luteus, Escherichia coli and Candida albicans,* as compared with other organisms tested. Finally 90µl/disc concentration showed greatest activity against *Klebsiella pneumoniae, Escherichia coli and Candida albicans.* Overall the methanolic extract showed greatest activity against *Klebsiella pneumoniae, Escherichia coli* and *Candida albicans,* This is in agreement with the previous reporters of same microbes with different medicinal plants(Diallo *et al.,*1999). From the antifungal activity of different concentrations (80, 90 and 100µl/disc) of methanolic extract of diacure showed greatest activity against *Aspergillus niger* and *Candida albicans* on the whole. Only 90µl/disc concentration showed greatest activity against *Aspergillus flavus*. In general, among the tested microbial strains, bacteria were found to be more sensitive to many of the test agents than fungi.

Conclusion

The processing of the plants performed in this study was not comparable to the traditional approach when the tribes used water extracts whereas we have used methanol extraction approach. Here it is not the exact replication of the traditional knowledge. All the same, methanol extracts use gives effective action against microbes, it is likely that water extracts will be effective as well and possibly mort so. The antibacterial activity of diacure, a polyhrebal formulation of 11 medicinal plants is reported for first time. No previous report on the antibacterial activity of these methanolic extract of diacure a polyherbal formulation of 11 medicinal plants could be found in the literature.

Among the concentrations tested 100µl/disc showed greatest antimicrobial activity indicating the potential for the discovery of antimicrobial principles. Several plants used by tribes exhibit some degree of antibacterial activity towards gram positive and gram negative bacteria. This dizcure could serve as useful source for new antimicrobial agents.

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