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Synergistic Antimicrobial Activity of *M.elengi* and *B. pinnatum* Plant Extract in Combination Against Microbes: An in Vitro Study

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Abstract*— Nowadays natural plant are possessing antimicrobial activity. So to support the sentence, number of studies were conducted to prove antimicrobial properties of B. pinnatum and M. Elengi with three different extracts ethanol, methanol and chloroform extracts against microbial pathogens. Several studies were done to support the antimicrobial properties of B. Pinnatum and M. Elengi but combination studies of both plants were not performed. Hence well diffusion assay and minimum bactericidal and fungicidal study was performed to check the antimicrobial properties in combination and it has been revealed that combination of both these plant extract can be an effective drug as compared to individual, which is supported by the data mentioned in the research paper.

Index Terms*— Plant Extracts, Antimicrobial Property, Minimum Inhibitory Concentration, Synergistic Activity

I. INTRODUCTION

Natural products are a source of synthetic and traditional herbal medicine and are still use in health care system worldwide [1]. Plants based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials need to occur. Modern-day pharmacopoeia however contains at least 25% drugs derived from plants. Involvement of medicinal plants as a re-budding health assistance has been powered with the rising burdens of prescription drugs in the treatment of personalized health and well-being along with the bio prospecting of new plant derived drugs [2]. Many reports have showed the effectiveness of traditional herbs against microorganisms; as a result plants are on the bedrocks for modern medicine to attain new principles [3].Since most of the bacteria are resistant to drugs so plant extracts are widely used for the treatment. Medicinal plants contain biologically active chemical substance such as alkaloids, glycosides, saponins, tannins, phenols and flavonoids which have curative properties. These complex chemical substances are known as secondary plant metabolites which are present in many plants [4]. There are several published reports describing the antimicrobial activity of various crude plant extracts either in combination or single [5].

In developing countries, there is a gradual revival of interest in the use of medicinal plants especially herbal preparations in the local healthcare systems because of the increasing problems of Multidrug Resistance (MDR) to human pathogenic bacteria [6]. The use of plant extract for medicinal treatment has become popular especially now when people are believing to realise that life span of antimicrobial is limited and misuse cause microbial resistance [7].

Bryophyllum Pinnatum (common name – Kalanchoe Pinnata) is one such medicinal plant which belongs to the Crassulacea family and it's usually brought up as life plant. *Bryophyllum pinnatum* is a succulent plant, 3-5 feet tall, 3.2cm wide, tall hollow stems, fleshy dark leaves that are rough distinctively, trimmed in red and bell-like nodding flowers. Medicative plants like *Bryophyllum pinnatum carries* with it phytochemicals with curative properties like tannins, saponins, triterpenes, steroids, alkaloids, flavonoids and alternative chemicals that are biologically active [8]. In ancient medication, the leaves juice is used for excretory organ stones treatment, applied against headache, cardiovascular disease, cancer, inflammation and wound infections and dandruff for scalp treatment [9].

Mimusops elengi (common name Bakul) is one such medicinal plant which belongs to family Sapotaceae, native to Western and Central Africa. *Mimusops elengi* is a small tree that grows in warm climates. It can reach heights of 30 foot and its leaves are pale on the underside and dark green on the upper side. *Mimusops elengi* also has flowers that usually appear yellow and green. In Ayurvedic medicine, *Mimusops elengi* is used to treat headaches, toothaches and oral diseases [10].

Traditional preparation of medicinal plants with antimicrobial activities have been extensively used worldwide [11]. Medicinal properties of *B Pinnatum* and *M Elengi* has been already studied and antimicrobial properties have been noted for *B Pinnatum* and *M. Elengi* [12]. Detailed study have been done for both the plants (*M elengi* and *B.pinnatum)* individually for assessing their phytochemical

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characteristics and compounds responsible for their antimicrobial properties. But present study is done to study the combine efficacy of both the plant extracts which have already depicted their antimicrobial properties alone. Combined therapy is traditionally used to increase antimicrobial activity and reduce toxic effects of agents [13].Therefore the present study was undertaken for the first time to investigate synergistic activity of *B pinnatum* and *M elengi* against microbes.

II. MATERIALS AND METHODS:

Plant collection and preparation of plant extracts:

Fresh healthy leaves, of B.Pinnatum and *M. elengi* were collected from Amravati district. The plant parts were cleaned and dried in shade for 7 days and then grinded well to fine powder. About 500 gm of dry powder of each plant were extracted with ethanol, methanol and chloroform (80%) at 70°C by continuous hot percolation using Soxhlet apparatus. The extraction was continued for 24 hrs and the ethanolic, methanolic and chloroform extract was then filtered and kept in hot air oven at 40 °C for 24 hrs to evaporate solvents ethanol, methanol and chloroform from it. A dark brown residue was obtained. The residue was kept separately in air tight containers and stored in deep freezer.

Collection of Bacterial and fungal samples:

The isolates include gram positive bacteria (*S.aureus*), gram negative bacteria (*E.coli*) and fungal culture (*Candida albicans*) which were procured from National Culture of Industrial Microorganisms (NCIM), Pune and after receiving the cultures gram staining and biochemical characterisation were done. After confirmation through biochemical test, sub culturing was done and inoculum were prepared for bacterial and fungal culture.

Standardisation of inoculum:

Bacterial and fungal suspension matching the turbidity of the 0.5 McFarland standards were prepared. The resulting suspension was then used as inoculum for the test pathogen to check the antimicrobial activity [14].

Determination of antimicrobial activity by agar well diffusion assay

Agar well-diffusion method was followed [15] to determine the in vitro antimicrobial synergistic activity of both the plant extracts. Nutrient agar (NA) and Muller Hinton agar (MHA) plates were swabbed (sterile cotton swabs were used) with 24 and 48-hour old broth culture with count of 100 cfu/ml of bacteria and fungi respectively. Four Wells (6mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer for *M.elengi* extracts, *B.pinnatum* extract, and (*B.pinnatum* + *M.elengi)* mixture extract and one for negative (solvent, DMSO) in which the particular extracts were diluted. Stock solution of each plant extracts individually and mixture of both plant extract were prepared at varying concentration (10, 5, 2.5, 1.25 mg/ml) in different solvents such as Methanol, Ethanol and chloroform. About 100 µl of plant solvent extracts individually and mixture of plant extracts of varying concentration were added with pipettes into the wells and allowed to diffuse at room temperature. Control experiments comprising inoculums without plant extract were set up to check the growth promotion of the organisms. The plates were then incubated at 37°C for 18-24 h for bacterial pathogens and 22°C for 48 hours for fungal pathogens. The diameter of the inhibition zone (mm) were measured for individual plant extract and plant extracts in combination.

Determination of minimum inhibitory concentration (MIC):

The fractions of individual leave extracts and combination of both plant extract that showed antibacterial and antifungal potential were further assessed for the minimum inhibitory concentration [16] which is the minimal concentration of plant extracts required to inhibit bacterial and fungal growth.

A stock solution of extract of two plant individually and in combination was prepared by dissolving 100 mg of each of the dried extracts in 5 ml of ethanol, methanol and chloroform and from this stock 2-fold serial dilution of 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg/ ml were prepared in Muller Hinton broth. This concentration of plant extracts were used for determination of minimum inhibitory concentration.

Broth dilution method was used for determination of minimum inhibitory concentration. Briefly 2 ml of nutrient broth and Muller Hinton broth were added into six test tubes and 0.1 ml of the prepared concentration of each extract individually and in combination were mixed with the nutrient broth and Muller Hinton broth respectively for bacteria and fungi respectively. Thereafter 0.1 ml of standardized inoculum of bacteria and fungi were dispensed into the test tube containing the suspension of broth and the extract. Then all test tubes were properly corked and incubated at 37°C for 24 hr for bacteria and at 22 °C for 48 hr for fungi. After which, they were observed for absence or presence of visible growth. The lowest concentration without visible growth of organisms was regarded as the MIC. Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) was done to assess the minimum quantity of plant extract required to kill bacteria and fungi growth respectively. Two control tubes were maintained for each test batch. These include tube-containing extract without inoculum and the tube containing the growth medium and inoculum.

III. RESULTS

In vitro antibacterial activity of *B.pinnatum* and *M.elengi* individual extract and mixture of both plants have been assessed by inhibitory zone diameter as given in Table 1 and 2 and Table 3 depicts MBC and MFC to check its minimum inhibitory concentration of plant extract individually and along with mixed plant extracts to justify its synergism.

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Table 1: Antimicrobial activities of *B.pinnatum* and *M.elengi* (Individually) against three M/O with zones of inhibition in mm using agar well diffusion method

Plant extracts	Concentration (mg/ml)	*E.coli*	*S.aureus*	*Candida albicans*
Ethanol extract				
B.Pinnatum	10	19	16	15
	5	10	11	12
	2.5	10	11	12
	1.25	-	-	-
M. Elengi	10	17	13	15
	5	10	11	10
	2.5	7	6	7
	1.25	-	-	-
Methanol extract				
B.pinnatum	10	20	18	18
	5	12	12	9
	2.5	8	8	6
	1.25	-	-	-
M.Elengi	10	20	20	18
	5	16	14	11
	2.5	9	9	6
	1.25	-	-	-
Chloroform extract				
B.pinnatum	10	16	18	18
	5	9	15	16
	2.5	8	9	8
	1.25	-	-	-
M.elengi	10	12	14	13
	5	9	10	8
	2.5	6	7	5
	1.25	-	-	-

Table 2: Antimicrobial activities of mixed extracts of *B.pinnatum* and *M. elengi* (Synergism) against three M/O with zones of inhibition in mm using agar well diffusion method

Plant extracts	Concentration (mg/ml)	E.coli	S.aureus	Candida albicans
Ethanol extract				
Mixed extracts (B pinnatum + M.elengi)	10	35	32	30
	5	24	26	25
	2.5	20	18	20
	1.25	6	5	5
Methanol extract				
Mixed extract (B.pinnatum + M.elengi	10	42	40	39
	5	30	28	21
	2.5	18	17	14
	1.25	5	7	5
Chloroform extract				
Mixed extract (B.pinnatum + M.elengi	10	30	32	32
	5	20	27	26
	2.5	15	15	15
	1.25	-	-	-

Table 3: Mean MBC and MFC (mg/ml) results of *B.pinnatum* and *M.elengi* individually of all extracts and along with mixed extracts with all solvents by broth dilution method

Plant extracts		E.coli	S.aureus	Candida albicans
B.pinnatum (Ethanol extract)	MBC	5.00	5.00	-
	MFC	-	-	5.00
B.pinnatum (Methanol extract)	MBC	5.00	5.00	-
	MFC	-	-	5.00
B.pinnatum (Chloroform extract)	MBC	10.00	10.00	-
	MFC	-	-	10.00
M.elengi (Ethanol extract)	MBC	5.00	5.00	-
	MFC	-	-	5.00
M.elengi (Methanol extract)	MBC	5.00	5.00	-
	MFC	-	-	5.00
M.elengi (Chloroform extract)	MBC	10.00	10.00	-
	MFC	-	-	10.00
Mixed extract (Ethanol)	MBC	0.625	0.625	-
	MFC	-	-	1.25
Mixed extract (Methanol)	MBC	0.625	0.625	-
	MFC	-	-	1.25
Mixed extract (Chloroform)	MBC	1.25	1.25	-
	MFC	-	-	2.5

IV. DISCUSSIONS

The objective of antimicrobial activity was to analyse past, present and future of medicinal plants to suggest there therapeutic activities. Recently several antibiotics have been used widely to inhibit bacterial and fungal growth. Several side effects of these substances and the development of antimicrobial resistant pathogens have become an ever increasing therapeutic problems. Many justify further research and development of natural antimicrobial agent. In the recent decade antimicrobial activity of plants in different areas of the world has been studied [17]. The zone of

inhibition study and MIC study reflected that both the plants (*B.pinnatum* and *M.Elengi*) had significant antimicrobial properties, [18] and [19]. After achieving significant antimicrobial activities form both the plants (*B.pinnatum* and *M.elengi*), it was thought to check its synergistic activity (to study in combination) and synergistic activity showed more zone of inhibition sizes as compared to that from the individual plant extracts. As shared from the results in above Table 1 and 2, mixed extract of *B. Pinnatum* and *M .Elengi* plant extracts with different solvent showed more inhibition diameter against microbes. After observing very good results in inhibition zone , minimum bactericidal and fungicidal experiments were carried out which showed that the minimum concentration required to inhibit the growth of bacteria and fungi from the mixed extract is much less as compared to the individual extracts obtained from different plants which is shown in Table 3.Individual studies were done on *B. pinnatum* and *M. Elengi* but combined studies of *B. Pinnatum* and *M. Elengi* to observe their synergistic effect was not carried out earlier. This study reveals that combination of both the plant extract showed inhibition activity more as compared to individual as depicted in the results above in Table 1, 2 and 3.

This study is supported with the study done with the mixture of Tribulus terrestis, Capsella Bursa Pastoris and G. Glabra against oral pathogens, were combination study has showed more inhibitory activity as compared to the individual extract [20]. Therefore this in vitro study provides scientific evidence to support uses of *B. pinnatum* and its combination with *M. Elengi* for the treatment of microbial infections.

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