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EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *MUTHUCHIPPI PARPAM* - A SIDDHA DRUG

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ABSTRACT

Muthuchippi parpam a reputed siddha drug is used for Piles, Cough, Bronchitis and Fistula. In the present study, antioxidant and antimicrobial activities of aqueous extract of *Muthuchippi parpam* was evaluated. The antioxidant activity was evaluated by using reducing power/FRAP (Ferric reducing antioxidant potential assay), Inhibition of DPPH radical, ABTS radical cation decolorisation assay, Total phenolic content (TPC), and *In vitro* anti-lipid peroxidation assay using TBARS. Significant antioxidant activity was observed in all these assays. The MIC (minimum inhibitory concentration) values of various concentrations of aqueous extract of *Muthuchippi parpam* against different bacteria were studied. *S. typhi* and *K pneumoniae* showed MIC at 3.125 mg/ml whereas, for *E coli* and *P.aeruginosai* MIC were 6.25 mg/ml for 12.5mg/ml respectively. *B. subtilis* did not show any growth at lowest concentration of 1.56 mg/ml. The MIC values of the various concentrations of aqueous extract of *Muthuchippi parpam* against different strains of fungi were tested. *Aspergillus fumigatus* showed MIC at 25 mg/ml whereas, *Aspergillus flavus* and *Aspergillus niger* showed MIC at 12.5 mg/ml each.

KEYWORDS

Muthuchippi parpam, *Aspergillus fumigatus*, *A.flavus* and *A.niger* Antioxidant.

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INTRODUCTON

The traditional medicine is widely used for various human ailments. The usage of herbal medicine could be even traced right from the beginning of mankind. Man tried to know about the plants around him to satisfy his basic needs such as food, shelter and clothing¹. The medicinal plant contains one or more active principles to cure the diseases². Traditional system of medicines has become significantly more

popular all over the globe because of the effective and curative nature for chronic diseases with less toxicity. *Muthuchippi parpam* is prepared by mixing the marine product Muthuchippi (*Pinctada margaritifera*) and the leaf Juice of *Justicia adhatoda* and *Vitex negundo*, rhizome juice of *Curculigo orchioides*. In the Siddha medicine *Muthuchippi parpam* is widely used for the treatment of Piles, Fistula, Emaciation, Cough and Bronchitis³.

MATERIAL AND METHODS

Preparation of samples

Muthuchippi parpam was procured from the Indian Medical Practitioners Co-operative Pharmacy and Stores Ltd. (IMPCOPS) Chennai. The drug powder (5mg/ml) was used for the preparation of the drug ranging from concentration of 1000µg/ml to 10µg/ml. This was further used to assess the antioxidant activity. The powder (200 mg/ml) was used for the preparation of the drug ranging from concentration of 100 mg/ml to 1.56 mg/ml. This was further used to assess the antimicrobial activity.

DETERMINATION OF ANTIOXIDANT BY VARIOUS ANTI-OXIDANT ASSAYS

Reducing power / FRAP (Ferric reducing antioxidant potential assay)

The reducing power of *Muthuchippi parpam* was determined according to the method of Oyaizu⁴. About 200 mg of plant extract in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 g for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. BHT was used as the reference material. All the tests were performed in triplicate and the graph were plotted with the average of three observations. The absorbance obtained was converted to BHT equivalent in mg per gm of dry material (BHT/g) using BHT standard curve.

Inhibition of DPPH radical

The free radical scavenging activity of *Muthuchippi parpam* was measured by 1, 1-diphenyl-2-picrylhydrazil (DPPH) using the method of Blois⁵ and 0.1 mm solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of various concentration of plant extracts and the reference compound (50, 100, 150, 200 and 250 µg). After 30 min, absorbance was measured at 517 nm. BHA was used as the reference material. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.

ABTS radical cation decolorisation assay

In this assay, the oxidant is generated by per sulfate oxidation of 2, 2'-azinobis (3-ethylbenzoline-6-sulfonic acid) - (ABTS²⁻) as described by Re *et al*⁶. ABTS radical cation (ABTS⁺) are produced by reacting ABTS solution (7mM) with 2.45 mM ammonium persulphate and the mixture was allowed to stand in dark at room temperature for 12-16 hrs before use. After 16 hrs, this solution was diluted with ethanol until the absorbance reaches 0.7 ± 0.02 at 734 nm. For the study, different concentration of *Muthuchippi parpam* was added to 0.3ml of ABTS solution and the final volume was made up with ethanol to make 1ml. The absorbance was read at 745nm and the percentage inhibition calculated.

Total Phenolic Content (TPC)

Total phenolic content (TPC) from *Muthuchippi parpam* was quantified using Folin - Ciocalteu's method adapted to 96-well micro titre plate with minor modifications⁷ 100 µl of 1:4 diluted Folin-Ciocalteu's phenol reagent, 2N (Sigma - Aldrich) in distilled water was added to 20 µl of serially diluted (10 - 1000 µg/ml) lyophilized plant extracts and standard gallic acid dissolved in distilled water. After 5 min incubation at room temperature, 80 µl of sodium carbonate (75 g/L) was added to each well. The 96 well plates was slightly shaken and incubated for 30 min at room temperature in the darkness. The absorbance was measured at 735 nm using ELISA plate reader. The assay was repeated thrice and Total phenolic contents (average of three) were expressed as BHT equivalent per gram of lyophilized extract.

In vitro anti-lipid peroxidation assay using TBARS

Thiobarbituric acid reactive species (TBARS) assay was performed as described by Aazza *et. al*⁸. Egg yolk homogenates were used as a lipid-rich medium obtained as described elsewhere⁹ briefly, 100 µl of egg yolk [(10% w/v) in KCl (1.15%)] and 50 µl of extract or standard vitamin E in ethanol (10 - 1000 µg/ ml) were added. Then, 300 µl of 20% acetic acid (pH 3.5) and 300 µl of 0.8% (w/v) TBA in 1.1% (w/v) sodium dodecyl sulphate (SDS) were added. The resulting mixture was vortexed and heated at 95°C for 60 min. After cooling, at room temperature, 750 µl of butan-1-ol was added to each tube; the contents of the tubes were stirred and centrifuged at 3000 rpm for 10 min. The upper organic layer was transferred to 96-well micro titre plate and absorbance was measured at 532 nm using an ELISA plate reader. All of the values were based on the percentage antioxidant index (AI%), whereby the control was completely peroxidized and each sample demonstrated a degree of change; the percentage inhibition was calculated using the formula $(1 - T/C) \times 100$, where C is the absorbance value of the fully oxidized control and T is the absorbance of the test sample. The antioxidant capacity was determined from three replicates. The percentage antioxidant index was plotted against the concentrations of samples and IC50 values were determined. Same amount of deionized water was used as the control.

Antimicrobial activity

Antibacterial Assays for Determination of Minimum Inhibitory Concentration (MIC) of the formulation:

The powder (200 mg/ml) was used for the preparation of the drug ranging from concentration of 100 mg/ml to 1.56 mg/ml. This was further used to assess the antimicrobial activity. Tetrazolium Micro plate Microbial viability Assay, a colorimetric assay based on the reduction of a tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) for rapidly determining the susceptibility of pathogenic strains to bactericidal ayurvedic drugs was carried out as described by Perumal *et al*¹⁰. Pathogenic strains of bacteria; *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhimurium*, *Escherichia coli*, *Klebsiella pneumonia* were used as test organisms. The aqueous

extract of *Muthuchippi parpam* was tested for their antibacterial potential, and results were noted down.

The 150 µl of drug was mixed with 150 µl bacterial culture in nutrient broth and then inoculated in the 96 well plates. Serial Dilutions were performed according to the protocol and kept for incubation at 37°C for 8 hours. The drug-free controls and appropriate solvent blanks were included as negative control. After the 8 hours incubation, cold 20% Tetrazolium solution was added to each well. The colour change was observed and noted for determining the MIC value of respective drug against the bacterial cultures. The bacterial growth was corresponded with the colour change to pink from the original colour of the respective drug and in absence of growth the colour remained the same. The MIC value was determined by observing the pink colour that indicates bacterial growth (+) and colourlessness that indicates inhibition of bacterial growth (-). The minimum concentration of the drug corresponded to the growth inhibition was treated as the 'MIC'.

Antifungal assay

Screening of antifungal activity of *Muthuchippi parpam*, against *A.flavus*, *A. fumigatus* and *A.niger* was also carried out. The drug and the fungal culture in the Sabouraud dextrose broth in the 96 well plate. Dilutions were performed according to the protocol and kept for incubation at 37°C for 5 days. Each day the fungal growth was observed to determine the MIC value of respective drug against three fungal pathogenic strains¹⁰. The MIC value was determined by observing the fungal plaques that indicated growth (+) and their absence indicated no growth (-). The following table gives an idea of the results that were obtained from this pattern.

RESULTS AND DISCUSSION

Ferric-reducing power (FRAP) assay

The *Muthuchippi parpam* showed to have 210µg BHT equivalent /1000µg of drug.

Inhibition of DPPH radical

The potential decrease in the concentration of *Muthuchippi parpam* DPPH radical due to the scavenging ability of BHA standard and aqueous extract showed significant DPPH scavenging activity

at 100 µg/ml concentration; 89.19% and 63.01% inhibition was observed for BHA and *Muthuchippi parpam* respectively.

ABTS radical cation scavenging activity

The potential decrease in the concentration of ABTS radical was due to the scavenging ability of Gallic acid standard and *Muthuchippi parpam* showed significant ABTS scavenging activity at 100 µg/ml concentration; 90.72% and 69.18% inhibition was observed for Gallic acid standard and *Muthuchippi parpam* respectively.

Total Phenolic Content

The *Muthuchippi parpam* 100 µg/ml extract contains 15.0943 µg BHT equivalent of total phenol.

In vitro anti-lipid peroxidation assay using TBARS

The potential anti-lipid peroxidation ability of Vitamin E standard and *Muthuchippi parpam* was observed. Thiobarbituric acid reactive substances scavenging activity for *Muthuchippi parpam* at 100 µg/ml

concentration was 51.82% and 47.17% inhibition was observed for Vitamin E.

Antibacterial Assays for Determining Minimum Inhibitory Concentration (MIC) of the formulation

The MIC values of the various concentrations for aqueous extract of *Muthuchippi parpam* against different bacteria is given in Table No.1. *S. typhi* and *K pneumoniae* showed MIC at 3.125 mg/ml whereas, for *E coli* it was 6.25 mg/ml and *P. aeruginosa* MIC was at 12.5mg/ml. *B. subtilis* did not show any growth at lowest concentration of 1.56 mg/ml.

Antifungal assay

The MIC values of the various concentrations for aqueous extract of *Muthuchippi parpam* against different strains of fungi is given in Table No.2. *Aspergillus fumigatus* showed MIC at 25 mg/ml whereas, *Aspergillus flavus* and *Aspergillus Niger* showed MIC at 12.5 mg/ml each.

Table No.1: Effect of aqueous extract of *Muthuchippi parpam* on different bacteria

S.No	Plant part	Minimum Inhibitory Concentration (MIC) in mg/ml				
		<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhimurium</i>	<i>Klebsiella pneumoniae</i>	<i>Bacillus subtilis</i>
1	-					
2	<i>Muthuchippi parpam</i> - aqueous extract	6.25	12.5	3.125	3.125	<1.56
3	Tetracycline Positive Control (µg/ml)	10.0	1.25	10.0	0.312	1.25

Table No.2: Effect of aqueous extract of *Muthuchippi parpam* on different strains of fungi

S.No	Plant part	Minimum Inhibitory Concentration (MIC) in mg/ml		
		<i>Aspergillus niger</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus flavus</i>
1	-			
2	<i>Muthuchippi parpam</i> aqueous extract	12.5	25	12.5
3	Amphotericin B Positive Control (µg/ml)	1.25	0.156	0.156

CONCLUSION

In this study, the aqueous extract of *Muthuchippi parpam* found to possess antioxidant and antimicrobial activities. The result of antioxidant evaluation based on five methods used in this study revealed that aqueous extract of *Muthuchippi parpam* possess significant antioxidant activity and remarkable antifungal activity. Hence, further evaluation has to be carried out to isolate the specific bioactive compound.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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