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PHYTOCHEMICAL ANALYSIS AND IN VITRO ANTIBACTERIAL AND ANTIFUNGAL STUDIES ON *PHAILERIA MACROCARPA (SCHEFF.) BOERL.* FRUITS

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Abstract

The objectives were to explore and evaluate the phytochemical, antibacterial and antifungal activity of crude extract of *Phaileria macrocarpa (Scheff.) Boerl.* (fruits mesocarp & pericarp) (CEPM) and it could be utilized in future to purpose a new line of treatment as antimicrobial agent. The plant sample was extracted by cold maceration with petroleum ether, chloroform, ethyl acetate and ethanol. The *in vitro* antibacterial and antifungal activities of the plant materials were determined by disc diffusion method and poisoned food technique respectively**.** The CEPM exhibited good antimicrobial (antibacterial & antifungal) effects against all tested microorganism. It demonstrated the highest zone of inhibition (20 mm) *E. coli* and *V. cholera* (2,000µg/disc). At the same time, it revealed the highest inhibition 55.17% of fungal radial mycelial growth against of *Aspergillus ustus* and *A. ochraceus* respectively (100µg/ml). The concentration were determined against *V. cholera with* (MIC,450µg/ml & MBC, 1000µg/ml)). However, for fungi (MIC700µg/ml & MFC, 1,500µg/ml) were recorded against *A. ochraceus*. The results of this study suggest that the active antimicrobial agent(s) are present in the CEPM; it may have potential for the treatment of bacterial and fungal infections.

Key words: Antimicrobial activity, *Phaileria macrocarpa,* Crude extracts, Spectroscopic.

Introduction

Therapeutic plants have turned into the center of extraordinary study regarding substantiation of their customary uses through the determination of their genuine pharmacological impacts furthermore inferable from a costly nature of manufactured medications and, potential symptoms [1-5]. Development of numerous medication imperviousness to human pathogenic living beings has likewise required a quest for new antimicrobial substances from different sources including restorative plants [6]. The substances that can either repress the development of pathogen or murder them and have no or slightest lethality to host cells are considered contender for growing new antimicrobial medications. Phytochemical examination has demonstrated that some plant inferred optional metabolites have powerful antimicrobial movement [7]. Among the diverse plant determined optional metabolites, alkaloids turned out to be the most vital gathering of exacerbates that demonstrated an extensive variety of antimicrobial movement [8].

Remedial utilization of plants proceeded with the advancement of progress and improvement of human learning [9-10]. Plants are still broadly utilized as a part of ethno medication around the globe [11-12]. In cutting edge China, the conventional drug construct chiefly with respect to the utilization of herbs is utilized generally as a huge instrument of human services [13]. Microorganisms have created imperviousness to numerous anti-infection agents and this has made monstrous clinical issue in the treatment of irresistible maladies [14]. This circumstance constrained researchers to look for new antimicrobial substances from different sources. Plant chiefly contains alkaloids, glycosides, sterols, D-mannitol and ursolic corrosive [14]. Vincristine to say a couple illustration of regular medication that have straightforwardly added to cutting edge drug. The quantity of possibly dynamic medications were gotten from plants simply numerous [15]. China and India have institutionalized their own particular indigenous pharmaceutical and pharmacopeia yet nations in Africa, in spite of the weights of infection and the plenitude of plant species, have not stuck to this same pattern [16].

Around 100 such medications of characterized structure are in like manner utilize today all through the world and about portion of them are acknowledged as valuable medications in the industrialized nations [17-18]. The Borreverine alkaloid extricated from this plant had an antimicrobial activity in vitro. The negligible inhibitory fixation is lower than 50 micrograms/ml for Gram positive cocci, (extraordinarily Staphylococcus aureus) and afterward 6 micrograms/ml for Vibrio cholerae and upper than 200 micrograms/ml for a few Gram negative bar microscopic organisms (Enterobacteria and Pseudomonas). These preparatory results underline the enthusiasm for the examination about the antimicrobial specialists from plant birthplace, specifically concerning actually or artificially altered alkaloids [19]. It becomes all over the place in Bangladesh as a weed in developed fields.

In light of the plant ethnopharmacological foundation specified over, the present examination was embraced to contemplate the plant concentrates and one of its built up unadulterated compound on different pathogenic bacterial and contagious strains, the consequences of which are being accounted for in the present studies.

Methods

Collection and Identification of plant material

The selected plant *Phaileria macrocarpa (Scheff.) Boerl.* fruit was collected in fresh condition from area of Indera Mahkota, Kuantan, Pahang, Malaysia in April 2013.

Extraction of plant material

The cleaned and collected samples were fragmented into small pieces (1-2 cm), air dried. The samples were grounded to produce fine powder mechanically and then 20 g of the dried powder was kept steeped 72 h in petroleum ether, chloroform, ethyl acetate and ethanol in different beaker [11]. On the other hand, 250 g of the dried powder was kept steeped 72 h in ethyl alcohol. Thus, the extracts were obtained separately, and then were filtered, centrifuged at 2,000 rpm for 20 min and finally concentrated to a gummy material under reduced pressure at 50˚C the remaining solvents were completely evaporated, employing rotary vacuum evaporator. Using water bath, the samples were dried and kept in small beaker.

Qualitative phytochemical screening of crude extract of Phaileria macrocarpa (Scheff.) Boerl.fruits

The testing of different chemical constituents in the extracts represents the preliminary phytochemical studies. The tests were performed as follows:

In every test 10% (w/v) arrangement of concentrate in methanol was taken, unless generally said in individual test. The accompanying tests were

performed for recognizing diverse compound gatherings.

Tests for alkaloids

Mayer's test

0.2 ml HCL of dilute and 2ml solution of the extract were taken into a test tube. After that, 1ml of Mayer's reagent was mixed together with the previous solution. Formation of yellow precipitate means the existence of alkaloids [20].

Dragendroff's test

2ml solution of the extract and 0.2ml of dilute HCL acid were taken in a test tube. Then 1ml of Dragendroff's reagent was taken with the previous solution. Orange brown precipitate was formed; this clearly demonstrates alkaloids is available in the solution [21].

Tests for Glycosides

Very little amount of an alcoholic extract was added to mixture of water and ethanol, afterwards they were heated with Fehling's solution. The presence of glycosides is indicated by Brick-red precipitate. Another portion of the extract was dissolved in water and alcohol, later they were heated with few drops of dilute H_2SO_4 that were neutralized with NaOH solution and finally heated with Fehling's solution. Brick-red precipitate was taken as a proof of the presence of glycosides [22].

Tests for Steroids

Sulphuric acids test

1ml solution of chloroform extract was taken and then mixed with 1ml H_2SO_4. Here, the nonappearance of red colouration clearly shows that steroids do not exist.

Tests for reducing sugars

Benedict's test

0.5ml extract of water of the plant materials was added in a test tube. Next, 5ml Benedict's solution was taken to the test tube, heated for 5 minutes and finally allowed to cool spontaneously. There is no sign of red color precipitate that displays that presence of reducing sugars.

Fehling's test (standard test)

2ml aqueous extract of the plant material was mixed with 1ml mixture of equal volumes of Fehling's solution A and B were heated for few minutes in the water bath. In sample A, a red or brick-red color precipitate was not formed, this indicates that reducing sugar was present. In sample B, on the other hand, absence of red or brick-red color precipitate indicates the nonexistence of reducing sugar.

Alpha Naphtha solution test

2 drops of sugar, 5ml solution of extract and 5% alpha naphtha solution (freshly prepared) are mixed and 1ml of sulfuric acid was added on the sides of the test tube. Violate colored ring was not seen at the junction of two liquids. This indicates that there is no reducing sugars [23].

Tests for Tannins

Ferric chloride test

5ml solution of the extract was taken in a test tube. Then 1ml of 5% ferric chloride solution was mixed. The presence of tannins is confirmed by the existence of greenish black precipitate [23]

Test for Flavonoids

Crude extract was dissolved in a minimum amount of methanol. To this a few drops of conc. Hydrochloric acid was added, immediately red colour developed. This test indicates the presence of Flavonoid [23-24].

Tests for Saponins

About 1ml solution of the extract was diluted with distilled water to 20ml and Shaken in a graduated cylinder for 15 minutes. Foams were observed, which indicates presence of saponins [25-26].

Incubation of the Plates and Determination of the Percentage of Inhibition

Every one of the plates (counting test, standard and control) were hatched at a temperature of 25±20C for 5 days, after which the outspread development of contagious settlement was measured with a straightforward scale in mm and the rate of hindrance of mycelial development was ascertained utilizing the accompanying mathematical statement [26]

$$I = \frac{C - T}{C} \times 100$$

Here,

I = Percentage of inhibition

C = Diameter of fungal colony in control

T = Diameter of fungal colony in treatment

Test Organism

Antibacterial and antifungal effects of *CEPM* were tested against eleven human pathogenic bacteria *viz- Shigella dysenteriae, Shigella sonnei, Salmonella typhi, Salmonella paratyphoid, Bacillus subtilis,*

Bacillus cereus, Bacillus megaterium, Staphylococcus aureus, Pseudomonas aeruginos, Escherichia coli and *Vibrio cholerae* and four human pathogenic fungi *viz- Aspergillus niger, Aspergillus ochraceus, Aspergillus Ustus* and *Candida albicans* composition of the media is describe in bellow.

NA Media (PH- 7.4 ± 0)

The in vitro antibacterial and antifungal exercises of the plant materials were controlled by plate dispersion technique [27] and harmed nourishment method separately. Mueller-Hinton (agar and soup) medium was utilized for society of microscopic organisms and Sabouraud (agar and juices) medium was utilized for society of growths. 5% ethanolic arrangement of the unrefined concentrates and immaculate compound were utilized as the test material. Every one of the outcomes were contrasted and the standard antibacterial medication Ampicillin and antifungal medication Clotrimazole. The base inhibitory fixation (MIC), least bactericidal focus (MBC) and least fungicidal focus (MFC) estimations of the rough concentrates were dictated by large scale weakening soup system [28].

Results

The crude extracts (chloroform, petroleum ether, ethanol and ethyl acetate extracts) taken from CEPM were evaluated to detect their antibacterial activity against eleven human pathogenic bacteria. The results of the sensitivity test were demonstrated in table 1-4.

In this study, the successive extracts of the fruits of the plant CEPM (*viz*. Ethanol extract) were evaluated to detect their antimicrobial property against human pathogenic bacteria (gram positive and gram negative), yeast (human pathogen). For antibacterial and anti-yeast screening, disc diffusion method was employed. The gram-positive bacteria were *Staphylococcus aureus*, while the gram-negative bacteria included Escherichia coli. The concentration of the extracts used was 1 mg per disc. A standard antibiotic disc of Amoxicillin (30 µg/disc) was utilized for making a comparison of antibacterial activity figure-1.

The extracts were tested for the antifungal properties against on human pathogenic yeast, *Candida albicans, Aspergillus Niger, Aspergillus Orchraceus.* For comparison of the activities, Clotrimazole (30µg/disc) was used as Standard. Results obtained from the screening are following table-5 The results showed that the antibacterial activity of the extracts was pretty good. Another important aspect to be mentioned here is that the extracts were not strongly active against gram-negative bacteria. None of the three extracts showed any activity against the yeasts tested while the standard antibiotic Clotrimazole showed very strong activity against them. Therefore, this means that the extracts have no anti-yeast properties.

Discussion

All the unrefined concentrates aside from petroleum ether extricate displayed great antibacterial movement against no less than seven bacterial strains tried. In any case, the ethyl acetic acid derivation and ethanol removes indicated similarly the better antibacterial action against all the eleven bacterial pathogens tried in this. The ethanol remove displayed the biggest zone of restraint (20 mm) with 1,000 µg/disc extract against *E. coli*

The antibacterial activity, MIC and MBC values of CEPM are presented in Table 2. It appeared that the crude extract exhibited good antibacterial activity against all the bacterial strains. The largest zone of inhibitions (20 mm and 15mm in diameter) was recorded against *V. cholerae* and Bacillus cereus with the crude extract at the concentration of 750 and 1,000µg/disc respectively. It exhibited the MIC and MBC values from 450 to 1,000 µg/ml and 750 to 1500µg/ml respectively. The lowest MIC (450µ g/ml) and MBC (1000 µg/ml) were found against *V. cholerae.* The standard antibacterial ampicillin (20 µ g/disc) was also found to be active against all the bacteria tested here in. The ethyl acetate and ethanol extract exhibited comparatively better antifungal activity than the others. The highest inhibition (55.17 %) of fungal radial mycelia growth was recorded against *A. ustus* with ethanol extract at a concentration of 100µg/ml medium. Antifungal activity; MICs and MBCs of CEPM are presented in Table 3. From the results, it appeared that the crude extract exhibited good antifungal activity against all the fungal pathogens tested herein. The highest inhibition (55.17 %) of fungal radial *mycelial* growth was recorded against *A. ochraceus* with crude extract at a concentration of 100 g/ml medium. The lowest MIC (450 g/ml) and MFC (750µ g/ml) were determined against *A. ochraceus*. Standard antifungal antibiotic Clotrimazole (100µ g/ml) exhibited inhibitions of radial mycelial growth of all the six fungi. The antifungal activities of the pure compound from other plants have also been reported previously [30].

The antibiotic Amoxicillin (30µg/disc) used as standard for comparison has shown very strong inhibition of the entire test organisms. The inhibition shown by the extracts were relatively smaller than

the standard but considering this is related to the extracts were crude substances containing just a very minute percentage of pure compounds (cf. Amoxicillin, a pure compound. Methanol extract of the plant showed low activity against the test organisms whereas the ethanol extract did not show any antibacterial activity. This study found that CEPM has a potential antibacterial and antifungal property. It may be used as a novel natural antibacterial and antifungal agent against a wide variety of infectious microorganism.

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Table-1 Table displays Minimum bactericidal and fungicidal inhibitory concentration with different kinds of solvents of CEPM.

Tested bacteria	Diameter of inhibition Zone in mm (CEPM, µg/disc)							
	Petroleum ether		Chloroform		Ethyl acetate		Ethanol	
	750	1500	750	1500	750	1500	750	1500
Pseudomonas aeruginosa	2	1	3	5	7	9	3	6
Bacillus subtilis	1	4	5	9	12	9	6	8
Shigella dysenteriae	4	4	8	7	9	12	4	9
Vibrio cholera	3	3	7	10	6	7	10	6
Bacillus megaterium	0	1	3	6	7	8	9	12
Bacillus cereus	2	4	3	5	7	7	11	10
Escherichia coli	4	2	2	1	7	9	9	14
Salmonella typhi	3	1	1	3	5	12	7	9
Staphylococcus aureus	6	3	4	5	4	6	4	11
Shigella sonnei	2	6	5	6	9	9	8	8
Salmonella paratyphoid	7	2	8	5	3	5	3	5

Table-2 shows the comparison of the inhibitory effects of CEPM and standard antimicrobial drug (Amoxicillin) with minimum bactericidal inhibitory concentration.

Bacterium	Diameter of zone of inhibitions in mm			MIC of CEPM (µg/ml)	MBC of CEPM (µg/ml)
	CEPM		Amoxicillin (30 µg/disc)		
	750	1500			
Shigella sonnei	9	10	17	450	750
Escherichia coli	5	6	8	1000	1000
Staphylococcus aureus	8	9	16	750	1000
Bacillus cereus	7	6	13	450	750
Bacillus megaterium	9	9	10	750	1000
Vibrio cholerae	9	15	11	450	750
Bacillus subtilis	5	8	9	450	1500
Shigella dysenteriae	3	5	14	1000	1000
Salmonella typhi	8	6	10	750	750
Salmonella paratyphi	5	7	9	750	1500

Table-3 shows the comparison of the percentage of inhibition of fungal mycelial growth between CEPM and Clotrimazole with minimum and bactericidal inhibitory concentration.

Tested fungus	% of inhibition of fungal mycelial growth		MIC (µg/ml) of CEPM	MFC(µg/ml) Of CEPM
	CEPM (100 µg/ml)	Clotrimazole* (100 µg/ml)		
Aspergillus ochraceus	42	46.5	450	750
Aspergillus niger	37.8	38.4	1000	1000
Aspergillus ustus	31.3	46	1000	1500
Candida albicans	39	30.5	750	750

**Standard antifungal activity*

Table 4: In *vitro* antibacterial activities of CEPM extracts after 48 hrs of incubation

Name of bacteria	Zones of inhibition (in mm)			Result
	Methanol extract (1mg/disc)	Ethyl Alcohol extract (1mg/disc)	Amoxicillin (30µg/disc)	
Staphylococcus aureus	1.3	Nil	2.90	55.17%
Escherichia coli	1.5	Nil	2.80	46.43%
Shigella dysenteriae	1.5	Nil	2.70	44.44%
Bacillus subtilis	1.4	Nil	3.00	53.33%

Table 5: Results for the Fungus

Name of the Fungus	Methanol (1mg/disc)	Ethanol (1mg/disc)	Clotrimazole (30µg/disc)
Candida albicans	No activity	No activity	Strong activity
Aspergillus Niger	No activity	No activity	Strong activity
Aspergillus ochraceus	No activity	No activity	Strong activity

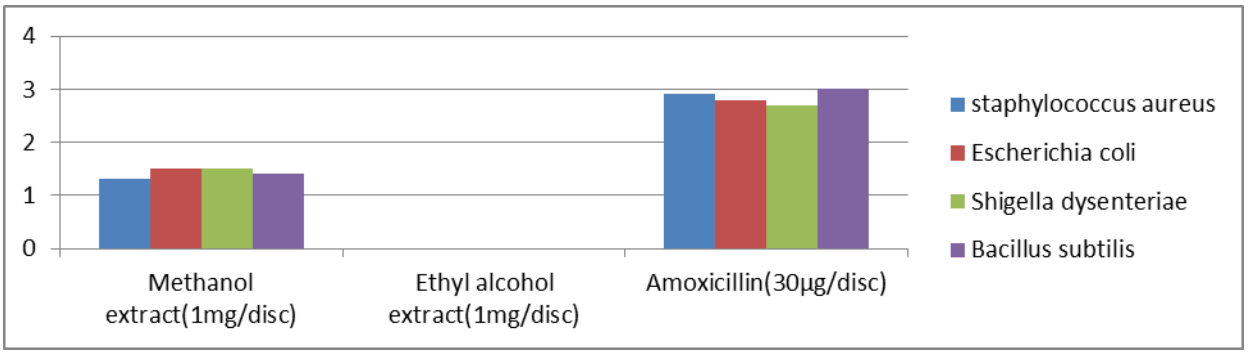

Figure-1 Comparison of antimicrobial activity of different media by the determination of zone of inhibition (mm)