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Research Article

Phytochemical Screening and Antifungal Potentials of Extracts of *Entada Africana*

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Abstract

The need to scientifically proof the traditional claim of the medicinal uses of plant materials in combating diseases has led to investigation into the medicinal potency of some plants. *Entada africana* (EA) stem bark has traditionally been used to treat microbial infections. This research was carried out to look at the medicinal potentials in the stem bark of the plant to seek its effectiveness against some diseases. The stem bark of *Entada africana* was air- dried, pulverized and macerated in methanol at ratio 1:5. Initial screening showed the plant to be rich in phytochemicals. The resulting methanol extract was further partitioned into alkaloid, flavonoid and saponin fractions. All fraction including the methanol extract was subjected to antifungal test to determine the best fraction with antifungal activity. Determination of antifungal activity was done using standard procedure and ketoconazole was used as the standard drug. The result of the antifungal test shows the flavonoid-rich fraction to possess the greatest antifungal activity as it was able to exert both fungicidal and fungistatic activity on the fungi isolates on which it was tested at a concentration of 200 mg/ml, saponin –rich fraction was next to it exerting fungistatic activity on all the fungi isolates and fungicidal on 4 of the fungi isolates on which it was tested. It is evident from this results that the flavonoid-rich fraction of EA possesses potent antifungal principles which lay credence to its use as treatment for microbial infections.

Keywords: Alkaloid, Flavonoid, Saponin-rich Fraction, Standard Drug, Fungistatic Activity

Introduction

Entada africana has been widely used traditionally to treat different ailments. It has been used as insecticides, source of gums in some small carpentry works [1]. Studies on the pharmacognostic characterization, nutritional, elemental and physicochemical contents had been reported [1]. *In vivo* and *in vitro* studies on the plant validated some ethnomedicinal claims of the use of the plant as an anti-inflammatory, analgesic, antibacterial, antioxidant, antiviral, anti-angiogenic, and anti-cytotoxic agents among others [1]. Triterpenes, saponins, flavonoids and sugars were reported as bioactive constituents which might be responsible for the aforementioned pharmacological actions of the plant.

Entada africana is a small tree which is mostly found in tropical and subtropical regions [2]. It is commonly known as "Tawatsa"in Hausa Language, "Ogurobe"in Yoruba. It can be used as food, medicine and fibre [1].

Natural products, such as plants extract, open a new horizon for the discovery of new therapeutic agents [3]. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed and about 80% of the world's population relies on herbal medicines [3]. Plants contain a wide range of chemical compounds that can be used to treat chronic as well as infectious diseases. They have been found to contain literally thousands of phytochemicals which have proved beneficial and have biological activity such as anticancer, antimicrobial, antioxidant, ant diarrheal, analgesic and wound healing activities [3]. Microbial resistance to the chemically synthesized drugs compelled us to move towards the ethnopharmacognosy. Thus, this study was carried out to identify some phytochemicals present in *Entada africana* and to evaluate its antifungal potentials.

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Materials and Methods

Materials

The materials and equipment include: rotary evaporator, hand gloves, tissue paper, weighing balance, conical flask, beaker, test tubes, test-tube rack, foil paper, paper tape, muslin cloth, measuring cylinder, water bath, separatory funnel, sampling bottle, conical flask, centrifuge, weighing balance, glass slides, cork borer, Potato dextrose agar plate, loop, broth medium, flask, spreader, pipette, incubator.

Reagents include absolute methanol, N-hexane, diethylether, hydrochloric acid, chloroform, soap, distilled water, N-butanol, potassium hydroxide, dilute HCl, alcohol, sterile saline and Agar. All reagents used were of analytical grade.

Sample collection

The E. *africana* stem bark samples were collected from a farm in Iwaro Oka Akoko, Ondo-State. They were later identified by Dr. Obembe of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The stem bark was shredded into pieces and air-dried for eight (8) weeks on cleaned ceramic slab surface and was later pulverized to powder.

Preparation of Methanol Extract

The *Entada Africana* stem barks were washed clean, air dried at room temperature and pulverized into fine powder using the milling machine to produce a homogenous sample and increasing the surface area. 2600 g of the powdered stem bark was macerated in 13 litres of absolute methanol and allowed to solubilize for 72 hours in which it was stirred intermittently. The mixture was sieved with sterile muslin clothe, the filtrate was then collected into a sterile container and left to form layers and further decanted to give the wet crude/methanol extract. The methanol mixture was further concentrated using the rotary evaporator to separate the solvent from the remaining plant residue at a given temperature below methanol's boiling point. Thereafter, the crude extract was air dried and the powdery substance obtained and preserved.

Phytochemical Screening

The crude extract was screened for the presence of some secondary metabolites such as saponins, alkaloids, and flavonoids using the methods described by Trease and Evans with slight modifications.

Test for Secondary Metabolites

The methanolic extract was screened for the presence of some secondary metabolites such as saponins, tannins, alkaloids, terpenoids, steroids, quinones, flavonoids and cardiac gycosides using the method of Sofowora [4]. Phytochemical screening involves performing simple chemical tests on the sample for the purpose of detecting different phytochemicals present.

Test for Alkaloids

0.5 g of crude powder was defatted with 5% ethyl ether for 15 minutes. The defatted sample was extracted for 20 minutes with 5ml of 1% hydrochroric acid (aqueous) in a steam bath. The solution was sieved through filter paper. Dragendorff´s reagent (1ml) was added to 1ml of filtrate. The mixture was observed for changes in the colour to black or formation of precipitate. The changes in colour indicates the presence of alkaloids.

Test for Saponins

The ability of saponins to produce fronthing in aqueous solutions was used as screening test for saponins. 2 g of the powder pearl millets was dissolved in distilled water in a test-tube and the mixture was shaken. Frothing which persist on warming was taken as preliminary evidence for the presence of saponins.

Test for Flavonoids

The presence of flavonoids in the sample was determined by the method described by Sofowora and Harborne [4]. 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of the extract followed by the addition of conc. H2SO4. A yellow coloration observed in the extract indicated the presence of flavonoids.

Test for Tannins.

0.5 g of the powder sample of pearl millets was boiled in 20 ml of distilled water in a test tube and then filtered.0.1% of ferric chloride was added. A blue-black or brownish green coloration was taken as evidence for the presence of tannin Trease and Evans.

Test for Terpenoids (Salwowski Test)

0.5 g of the extract was dissolved in 2 ml of chloroform, after which 3 ml of conc. H2SO4 was carefully added to form a layer. A reddish brown coloration at the interface indicated the presence of terpenoids.

Test for Steroids

0.5 g of the methanol extract of the sample was added to 2 ml of acetic anhydrides with 2 ml of H2SO4. No colour generation indicated absence of steroids.

Test for Cardiac Glycosides (Keller-Killani Test)

The extract 0.5 g was dissolved in 2 ml of glacial acetic acid containing one drop of 10 % (v/v %) ferric chloride solution. Concentrated sulphuric acid, 1 ml, was carefully added into the solution. A brown ring at the interface indicated the presence of deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer, a greenish ring may appear just above the brown ring and gradually spread throughout this layer confirming the presence of cardenolides.

Partitioning

Preparation of Alkaloid-rich Extract

The crude extract was separated from the neutral, acidic and water molecules by using 5% aqueous acetic acid (CH3CO2H) and was then filtered in which the filtrate obtained was extracted using dichloromethane in the separating funnel to get two distinct layer called the organic and aqueous phase. The organic phase was discarded and the aqueous phase was then basified with aqueous Na2CO3, 10% to pH of 10 in which a further extraction with dichloromethane was done.

Fractionation of Flavonoid-rich and Saponin-rich Extracts

The crude extracts was extracted with n-hexane giving an n-hexane portion and marc. The marc was then extracted using methanol in which a methanol portion and marc was derived. The methanol portion derived was then diluted with water and partitioned with diethyl ether in the ratio 1:5 and then left to stand overnight in a separating funnel. A diethyl ethyl portion and an aqueous portion was then retrieved the next morning. The aqueous portion was then partitioned with the n-butanol in the separating funnel and then left overnight to partition two distinct layers; n-butanol and the residual aqueous portion was retrieved in the morning. The n-butanol was then partitioned

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with 1% KOH (potassium hydroxide) to produce the n-butanol saponin fraction and a KOH portion.

The KOH portion was further acidified with dilute HCL and partitioned with n-butanol saturated with water. An HCL portion and n-butanol portion was then derived. The flavonoid fraction was obtained from the n-butanol portion.

Procedure for Antifungal Test

The wells were made using cork borer on potato dextrose agar plate. The borer was deep into the alcohol for sterilization and then was used to make wells. Plates were used for the zone of inhibition. Five well-isolated colonies of the fungus were selected from an agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4-5 ml of PDA broth medium. The broth culture is incubated at 350 C until it achieves turbidity 1-2 x 108 cells/ml. The turbidity of actively growing broth culture was adjusted with sterile saline.

Inoculum of Test Plates

Optimally, within 15 minutes after adjusting the turbidity of the inoculum's suspension, loopful of suspension inoculates into flask contains Agar. Mix it well and pour it into plate and rotate the plate for even distribution. On the dry PDA agar plate loopful suspension evenly spreaded by spreader.

Inoculums of Crude and Purified Components of Plant Extracts into the Well

i. In the plate, wells were made for the inoculation of metal complex. Minimum of 4 wells were made in one plate.
ii. Using sterile pipette, 200 mg/ml of crude and purified components of plant extracts and antifungal control drug was added into respective wells.
iii.The plates were first placed at 4°c for 30 minutes in order to allow the diffusion of the metal complex compound and antifungal drug.
iv.Then plates were incubated at 37°c for 24 hours at room temperature.
v.The diameter of the inhibition zones were measured in millimeter at the end of the incubation time.

Determination of Minimal Inhibitory Concentration (MIC)

Dilution susceptibility testing method was used to determine the minimal concentration of antifungal to inhibit or kill the fungus. This was achieved by dilution of antifungal to inhibit or kill the fungus and was achieved by dilution of antifungal in either agar or broth media (PDA).
Procedure for Performing the Minimum Inhibitory Concentration Test Inoculums Preparation

Inoculum's preparation was performed as discuss earlier in well diffusion method.

2. Procedure

i. Different concentration of metal complex in (pipette, 200 mg/ml to 25 mg/ml) of crude and purified components of plant extracts to the tube to respective tubes were added.

ii. From the inoculums 5 µl of each culture was inoculated separately in each set so that final concentration of fungus in tubes became 106 cells/ml. This procedure was performed for all the pipette, 200 mg/ml to 25 mg/ml of crude and purified components of plant extracts with antifungal activity which was obtained by primary screening.

iii. Then all sets of tubes of dilution broth were incubated at 37°c for 24 hours in incubator.
All sets of wells were observed for determination of MIC to the susceptible fungus were tested and note down the results also MBC (minimum bactericidal concentration) was carried from mic result.

Results

$$\text{Percentage yield of crude extract} = \frac{\text{Weight of crude extract}}{\text{Weight of ground sample}} \times 100$$

$$= \frac{300\ g}{2600\ g} \times 100$$

Percentage yield of crude extract = 11.54
The table below shows the result of the phytochemical screening of the methanolic stem extract. The analysis revealed the presence of high levels of flavonoids, saponins, tannins and alkaloids. Steroids and terpenoids were present in moderate amount.

Table 1: Phytochemical screening results

Phytochemical	Qualitative content of phytochemicals				
Test organisms	Flavonoids	Crude extract (mm)	Alkaloids (mm)	Saponins (mm)	ketoconazole (mm)
Mucor hiemalis	22	10	11	17	24
Aspergillus fumigatus	18	10	16	16	22

Flavonoids	+++
Saponins	+++
Tannins	+++
Steroids	++
Alkaloids	+++
Terpenoids	++

Key: (+++) = High content, (++) = Moderate content

Table 2: Effects of EA extracts on the various fungi isolates using the zones of inhibition in (mm) of compounds (250 mg/ml).

Aspergillus niger	20	16	16	15	24
Candida albican	36	19	21	24	30
Fusarium verticilliodes	16	10	12	14	21

Sensitive (s) = 21 mm above, intermediate (i) = 20- 15 mm, resistant (r) = less than 15 mm

Table 3: Minimum inhibitory concentration of flavonoid compounds (250 mg/ml) against various fungi isolates.

Test organisms	200 mg	150 mg	100 mg	50 mg	25 mg
Mucor hiemalis	+	+	-	-	-
Aspergillus fumigatus	+	+	-	-	-
Aspergillus niger	+	+	-	-	-
Candida albican	+	+	+	+	-
Fusarium verticilliodes	+	-	-	-	-

Key:
Positive = sensitive
Negative = not sensitive

Table 4: Minimum fungicidal concentration of flavonoid compounds (250 mg/ml) against various fungi isolates.

Test organisms	200 mg	150 mg	100 mg	50 mg	25 mg
Mucor hiemalis	+	-	-	-	-
Aspergillus fumigatus	+	-	-	-	-
Aspergillus niger	+	+	-	-	-
Candida albican	+	+	-	-	-
Fusarium verticilliodes	+	-	-	-	-

Key:
Positive = sensitive
Negative = not sensitive

Table 5: Minimum inhibitory concentration of saponin compounds (250 mg/ml) against various fungi isolates.

Test organisms	200 mg	150 mg	100 mg	50 mg	25 mg
Mucor hiemalis	+	+	-	-	-
Aspergillus fumigatus	+	-	-	-	-…
Aspergillus niger	+	-	-	-	-
Candida albican	+	+	+	+	-
Fusarium verticilliodes	-	-	-	-	-

Key:
Positive = sensitive
Negative = not sensitive

Table 6: Minimum fungicidal concentration of saponin-rich extract (250 mg/ml) against various fungi isolates.

Test organisms	200 mg	150 mg	100 mg	50 mg	25 mg
Mucor hiemalis	+	-	-	-	-
Aspergillus fumigatus	+	-	-	-	-
Aspergillus niger	+	+	-	-	-
Candida albicans	+	+	-	-	-
Fusarium verticilliodes	+	-	-	-	-

Key:
Positive = sensitive
Negative = not sensitive

Table 7: Minimum inhibitory concentration of alkaloid compounds (250 mg/ml) against various fungi isolates.

Test organisms	200 mg	150 mg	100 mg	50 mg	25 mg
Mucor hiemalis	+	+	-	-	-
Aspergillus fumigatus	+	-	-	-	-
Aspergillus niger	+	-	-	-	-
Candida albican	+	+	+	+	-
Fusarium verticilliodes	-	-	-	-	-

Key:
Positive = sensitive
Negative = not sensitive

Table 8: Minimum fungicidal concentration of alkaloid compounds (250 mg/ml) against various fungi isolates.

Test organisms	200 mg	150 mg	100 mg	50 mg	25 mg
Mucor hiemalis	-	-	-	-	-
Aspergillus fumigatus	-	-	-	-	-
Aspergillus niger	-	-	-	-	-
Candida albican	+	+	+	-	-
Fusarium verticilliodes	+	-	-	-	-

Key:
Positive = sensitive
Negative = not sensitive

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Table 9: Minimum inhibitory concentration of crude compounds (250 mg/ml) against various fungi isolates.

Test organisms	200 mg	150 mg	100 mg	50 mg	25 mg
Mucor hiemalis	-	-	-	-	-
Aspergillus fumigatus	-	-	-	-	-
Aspergillus niger	-	-	-	-	-
Candida albican	+	+	-	-	-
Fusarium verticilliodes	-	-	-	-	-

Key:
Positive = sensitive
Negative = not sensitive

Table 10: Minimum fungicidal concentration of crude compounds (250 mg/ml) against various fungi isolates.

Test organisms	200 mg	150 mg	100 mg	50 mg	25 mg
Mucor hiemalis	-	-	-	-	-
Aspergillus fumigatus	-	-	-	-	-
Aspergillus niger	-	-	-	-	-
Candida albican	+	-	-	-	-
Fusarium verticilliodes	-	-	-	-	-

Key:
Positive = sensitive
Negative = not sensitive

Discussion

Plant extracts for medicinal usage contain a variety of bioactive compounds. These compounds consist of multi component mixtures. Medicinal plants contain a variety of biologically active, non-nutritive compounds known as phytochemical, which impart health benefits such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property [5].

Extraction is the important step to recover and isolate bioactive compounds from plant materials. Efficiency of the extraction is strongly affected by the extraction method, temperature, extraction time, the composition of phytochemicals and the solvent used [6]. The percentage yield of crude extract was 1.87%

Preliminary phytochemical screening of the crude extract of the stem bark of E. *africana* presented in Table 1 revealed several polar and non-polar chemical constituents. It showed the high presence of alkaloids, flavonoids, tannins and saponins and also the moderate presence of steroids and terpenoids. The phytochemicals present in this plant gives credence to the use as therapeutic agent against microbes.

The phytochemical results revealed that the plants contained bioactive agents which are connected with antimicrobial properties in plants [7]. Flavonoids are reported to have antibacterial, anti-inflammatory, anticancer, antifungal, antiallergic, and diuretic properties [8]. Tannins are known to possess general antimicrobial and antioxidant activities. Saponins have been implicated as bioactive antibacterial agent of plants [4,9]. Alkaloids are medicinal agents for their analgesic antiplasmodic of disorders, and are known for their anticancer effects [10].

Nature has been the source of medicinal agents for thousands of years and since the beginning of man. In Nigeria, medicinal plant-based industry is growing annually for pharmaceuticals, phytochemicals, nutraceuticals, cosmetics and other valuable products. The phytochemical screening of E. *africana* revealed the high presence of flavonoids which are of medicinal interest and signifies that the E. *africana* plant is a flavonoid-rich plant. The results obtained in this study suggest that the high level of flavonoids is responsible for the efficacy of the plants parts of the species studied. The presence of some of these compounds has also been confirmed to have antimicrobial activity.

Flavonoids or bioflavonoids, are a category of secondary metabolites of plants and fungal origin. They are composed of a wide-range of polyphe¬nolic entities having a benzo-□-pyrone system and are pervasive in plants. Because flavonoids are second¬ary metabolites (biosynthesized through shikimic acid pathway) of plants, they are consumed by man via food too [11]. Flavonoids are polyhydroxyphenols which are synthesized by plants to act against microbial infec¬tions [12]. They are known to be responsible for antioxidant, anticancer, anti-inflammatory, hepatoprotective potential and antibacterial activity [13-16].

Recently, epidemiological studies have strongly suggested that consumption of plant flavonoids contributes to reducing the risk or incidence of some cancers. Also, more and more attention has been focused on the antioxidant activity of medicinal plants, fruits and vegetables that contain plentiful flavonoid compounds. However, the content of flavonoid compounds is influenced by many factors, including the genus, the place of the plant growth, the extraction condition and technology [17]

The results of this study show clearly that phytochemicals from *Entada africana* bark extract have effective antifungal potentials. Ketoconazole was used as standard antifungal drug and the crude extract of *Entada africana* stem bark which contains phytochemicals was also used as test compound. Phytochemicals isolated from *Entada africana* stem bark which include flavonoids, saponins and alkaloids showed potent antifungal activities.

Tables 2 shows the zone of inhibition for the different fractions of Ea against some fungi isolates. Flavonoid-rich fraction was seen to possess the best antifungal potential, inhibiting the fungi growths at zones similar to the standard drug ketoconazole. For *Mucor hiemalis*, it inhibited the growth of the fungi at zone of 22 mm while the standard did that at 24 mm. For *Aspergillus fumigatus*, flavonoid-rich fraction's zone of inhibition stood at 18 mm while that of ketoconazole was 22 mm; for *Aspergillus niger*, the zone of inhibition was noted to be 20 mm while for the standard drug it stood at 24 mm. *Candida albicans* had the highest zone of inhibition (36 mm) against the fungi when compared to the standard drug (30 mm) while

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fusarium venticilliodes had a zone of inhibition of 16 mm for flavonoid-rich against 21 mm for ketoconazole. For the other fractions they are either resistant or mildly sensitive to Mucor hiemales, *Aspergillus fumigatus*, *Aspergillus niger* &Fusarium verticilliodes but the alkaloid & saponin fraction were sensitive to the *Candida albicans*. This result was in agreement [18].

Table 3 indicates that minimum inhibitory concentration result is the combination of fungicidal and fungistatic.activities. Fungicidal activity has ability to kill totally while fungistatic stops growth and does not kill. Positve (+) indicates sensitivity and negative (+) indicates non-sensitivity. The positive tends to be either fungicidal or fungistatic. Fungi isolates used are *Mucor hiemalis*, *Aspergillus fumigatus*, *Aspergillus niger, Candida albicans, Fusarium verticilliodes* were all positive at 200 mg concentration of flavonoid-rich fraction respectively which makes them sensitive at this concentration. At 150 mg concentration of flavonoid-rich fraction, all test organisms show positive indicating sensitivity. Only, Fusarium verticilliodes showed nonsensitivity at this concentration. Flavonoid-rich fraction at 100 mg and 50 mg concentration, all test organisms showed nonsensitivity and *Candida albicans* was sensitive at this concentration. At 25 mg concentration of flavonoid- rich fraction, all test organisms were insensitive. This results was in agreement [18].

Table 4 shows the concentration at which fungi isolates were killed totally. At 200 mg concentration of flavonoid-rich fraction, all the isolates were are sensitive whereas, at 150 mg, only *Candida albicans* and *Aspergillus niger* were sensitive. At 100 mg, 50 mg, 25 mg concentrations of flavonoid-rich fraction, all the test organisms were not sensitive. This result was in agreement [18].

At 200 mg concentration of saponin-rich fraction, all the test organisms showed sensitivity except *Fusarium verticilliodes* which was not sensitive. However, at 150 mg concentration, *Candida albicans* and *Mucor hiemalis* were sensitive while others were not. Whereas, 100 mg and 50 mg concentrations were sensitive to *Candida albicans* and not sensitive to others. At 25 mg concentration, all test organisms used were not sensitive. As indicated in Table 6, at 200 mg of concentration of saponin-rich fraction, all test organisms used were sensitive.

At 150 mg of concentration of saponin-rich fraction, *Aspergillus niger*, *Candida albicans* were positive (+), showing they were sensitive while others were negative indicating they were not sensitive.

Using 100 mg, 50 mg, 25 mg of concentration of saponin-rich fraction, all test organisms used as fungi isolate were negative, indicating they were not sensitive.

Minimum Inhibitory concentration result in Table 3.5.1 Test organism used are *Mucor hiemalis*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans, Fusarium verticilliodes*.

At 200 mg concentration of alkaloid-rich fraction all test organism used as fungal isolate are positive which shows is sensitive at this concentration except *Fusarium verticilliodes,* which is negative indicating is not sensitive at 200 mg concentration of alkaloid-rich fraction.

At 150 mg concentration of alkaloid-rich fraction, *Aspergillus fumigatus*, *Aspergillus niger*, Fusarium verticilliodes are negative indicate is not sensitive while *Candida albicans* and *Mucor hiemalis* are positive indicating they are sensitive.

At 100 mg and 50 mg concentration of alkaloid-rich fraction, only *Candida albican* is positive indicating it is sensitive, all other test organism used as fungi isolate are Negative indicating they are not sensitive.
At 25mg concentration of Alkaloid-rich fraction,all are Negative showing none is sensitive.

Table 5 shows that *Mucor hiemalis*, *Aspergillus fumigatus*, *Aspergillus niger* is Negative at all concentration of alkaloids which includes 200 mg, 150 mg, 100 mg, 50mg, 25 mg respectively, showing non-sensitivity.

Candida albicans is positive at 200 mg, 150 mg, 100 mg concentration of alkaloid-rich fraction respectively, indicating that it is sensitive and it is negative at 50 mg and 25 mg.

Fusarium verticilliodes is negative at 150 mg ,100 mg, 50 mg, 25 mg concentration of alkaloid-rich fraction indicating it is not sensitive to alkaloid at this concentration, but at 200 mg it is positive which indicate that at alkaloid-rich fraction concentration it is sensitive.

Table 6 shows that *Mucor hiemalis, Aspergillus fumigatus, Aspergillus niger, Fusarium verticilliodes* is negative at all concentration (200 mg ,150 mg, 100 mg, 50 mg, 25 mg) of crude extract which indicate is not sensitive.

Candida albican is positive at 200 mg and 150 mg concentration of crude extract which indicate is sensitive and at 100 mg, 50 mg and 25 mg concentration of crude extract is negative and indicate is not sensitive at this concentration respectively.

Table 7 shows that at 200 mg concentration of crude extract all test organism (*Mucor hiemalis, Aspergillus fumigatus, Aspergillus niger, Fusarium verticilliodes)* used are negative, indicating they are not sensitive except *Candida albicans* which test positive, indicating it is sensitive to crude extract at 200 mg concentration.

At 150 mg,100 mg, 50 mg, 25 mg concentration of crude extract all test organism (*Mucor hiemalis*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albican, Fusarium verticilliodes)* used as fungus isolate are negative, indicating they are not sensitive

Conclusion

The results for antifungal tests show that the flavonoid-rich and saponin-rich fractions possess significant antifungal activity against all the fungi isolates but the best antifungal activity was demonstrated by the flavonoid-rich fraction. This shows that the flavonoid-rich can be further developed into antifungal drug and also characterization should be done to know the active compounds present in this fraction.

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