PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL ANALYSIS OF FOSSOMBRONIA INDICA STEPH.

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ABSTRACT

The research focused on conducting phytochemical screening and assessing the antioxidant and antimicrobial properties of Fossombronia indica Steph. The objectives were to identify bioactive compounds, compare their antioxidant capabilities with a standard antioxidant and evaluate their antimicrobial potential using various extracts against selected organisms. The study involved the use of n-hexane, ethyl acetate, methanol, and aqueous as solvents to extract the phytochemicals and subject the fractions antioxidant and antimicrobial analysis. The assessment of antioxidant properties included the2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, Ferric ion Reducing Antioxidant Power (FRAP), and the determination of Total Antioxidant Capacity (TAC), all compared to ascorbic acid as a standard antioxidant. The Agar well diffusion assay was utilized for testing the antimicrobial activity of six bacteria; Aeromonas hydrophylla, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, and Staphylococcus aureus, as well as two fungi; Aspergillus niger and Candida albicans. Statistical analysis involves the use of descriptive statistics and Analysis of Variance (ANOVA). In the phytochemical screening, the investigation of bioactive compounds revealed the presence of saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, terpenoids, steroids, alkaloids, and phenols. The results of the DPPH, FRAP and TAC assays indicated that F. indica showed significant antioxidant activity. Among them, the n-hexane fraction displayed the highest activity (28.9%) of DPPH at a concentration of 1000µg/ml. The ethyl acetate fraction of F. indicahas the highest value of FRAP (11.12) at a concentration of 1000µg/ml while the ethyl acetate fraction of F. indica exhibited the highest TAC activity, with a value of 2.834, at a concentration of 1000µg/ml. The antimicrobial results showed that the ethyl acetate fractions of F. indica at three different concentrations exhibited antibacterial high activity against all tested bacteria, with the exceptions of E. coli and S. aureus. The methanol fractions displayed antibacterial activity against all tested bacteria except A. hydrophila. Among them, the ethyl acetate fraction was particularly effective against P. aeruginosa and A. hydrophila. The methanol fractions inhibited the growth of all tested organisms except for E. coli. In the case of antifungal activity, C. albicans proved to be sensitive to all fractions of n-hexane, ethyl acetate, methanol, and aqueous fractions of F. indica. Overall, the study indicated that Fossombronia indica is rich in phytochemicals, possesses significant antioxidant and can inhibit the growth of microorganisms. Hence, can be recommended for the production of drugs.

Keywords: Antimicrobial, Antioxidant, Bioactive Compound, Bryophytes, Phytochemical

1.INTRODUCTION

With the quest for natural products, there has been an increased focus on exploring various avenues to discover secondary metabolites in plants. This interest in plant-based alternatives has become imperative in light of the ongoing development of drug resistance in pathogenic microorganisms (Edewor et al., 2015). Researchers have noted that bryophytes contain a wide array of phytochemical compounds with diverse biological activities (Alam, 2012; Isa et al., 2014). Among the phytochemicals found in these plants, both primary and secondary metabolites have been identified, including alkaloids, phenolic compounds, anthraquinones, tannins, saponins, glycosides, terpenoids, polyphenols, amino acids, fatty acids, and sterols, among others (Mishra et al., 2014; Klavina et al., 2015). These compounds have demonstrated various beneficial properties, such as antibacterial, anti-inflammatory, anticancer, antitumor, and cytotoxic activities (Ivanova et al., 2007; Shi et al., 2008; Shi et al., 2009). For instance, *Plagiochasma japonica* and *Marchantia tosana* have been reported to exhibit antitumor, antibacterial, antifungal, muscle relaxation, thrombin activity inhibition, and superoxide release inhibition (Lahlou et al., 2000).

While the human body naturally produces antioxidants, this defense mechanism is not completely effective, especially in the face of excessive free radical production, and its effectiveness tends to decline with age. Free radicals can cause damage to biological molecules in the body, leading to issues such as DNA damage, damage to membrane lipids, cellular impairment, and ultimately contributing to the development of diseases like cancer, cardiovascular disease, rheumatoid arthritis, diabetes, and neurological disorders (Valko et al., 2007).

Given the limited exploration of bryophytes due to their small size, there remains less exploration regarding their phytochemical activity, antimicrobial properties, and antioxidant potential. In light of the growing resistance of microorganisms to conventional drugs, it has become essential to investigate the potential of bryophytes as antimicrobial agents in the development of antibiotics. This study was conducted to analyse the phytochemical constituents, evaluate the antioxidant potential and assess the antimicrobial activityof*Fossombronia indica* Steph

2. MATERIALS AND METHODS

Collection and processing of plant material

Fossombronia indica was collected from the Botanical gardens $(07^{\circ} 23' 28'' \text{ N}, 03^{\circ} 54' 59'' \text{ E})$ and the Zoology department $(07^{\circ} 26' 44'' \text{ N}, 03^{\circ} 53' 44'' \text{ E})$ University of Ibadan, Ibadan, Oyo state, Nigeria. They were identified at the Department of Botany, University of Ibadan, Oyo state, Nigeria. They were processed according to the method of Oyesiku and Caleb (2015).

Solvent Extraction of the Bryophyte

The sample was subjected to a series of sequential solvent extractions, progressing from nonpolar to polar solvents (from n-hexane, followed by ethyl acetate, methanol, and aqueous), in order to extract a diverse spectrum of compounds, following the methodology outlined in Das et al. (2010)

The samples were subjected to successive solvent extraction using solvents of increasing polarity from non-polar to polar (n-hexane, ethyl acetate, methanol and aqueous) respectively to extract wide range of compounds (Das et al., 2010)

Phytochemical Analysis of the Bryophyte

Qualitative and quantitative assessments were conducted to confirm the existence of various phytochemicals, following the methodologies detailed in Hussain et al. (2011)

Determination of Antioxidant Activity of the Bryophyte

The assessment of the fractions' capacity to scavenge free radicals followed the method outlined in Manzocco et al. (1998) and Ayoola et al. (2006). The determination of Ferric Reducing Antioxidant Power (FRAP) in the fractions were conducted following the methodology of Benzie and Strain (1999). Additionally, the Total Antioxidant Capacity (TAC) of the fractions was evaluated using the approach described by Prieto et al. (1999)

Antimicrobial Analysis of the Bryophyte

Isolates of various organisms, including *Aeromonas hydrophila*, *Bacillus subtilis*, *Escherichia coli*,*Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus* (bacteria), were obtained from the University College Hospital in Ibadan. Additionally, *Aspergillusniger*(fungus) was isolated from *Allium cepa*, and *Candida albicans* was collected from UCH, Ibadan. These isolates were reactivated by culturing them on Nutrient Agar and Potato Dextrose Agar, respectively, before being used for experimentation.

To assess antibacterial and antifungal activity, different fractions and three concentrations (100, 50, and 25 mg/mL) of a 10% DMSO solution containing bryophyte extracts were subjected to a standard Agar well diffusion assay, following the procedure outlined by Perez et al. (1990). As positive controls for bacteria and fungi, $5\mu g$ of ciprofloxacin, $10 \mu g$ of streptomycin, and 200 μl of nystatin (5mg/ml) were employed. The experiments were duplicated, and the resulting mean values were calculated, adhering to the methods described by Sawant et al. (2010) and Ulka et al. (2010).

Isolates of the following organisms, *Aeromonas hydrophila*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus* (Bacteria) were collected from University College Hospital, Ibadan. The fungi, *Aspergillus niger* was isolated from *Allium cepa* and *Candida albicans* was collected from UCH, Ibadan. They were resuscitated on Nutrient Agar and Potato Dextrose Agar respectively prior to use. The different fractions and three concentrations (100, 50 and 25 mg/mL) of 10% DMSO solution of the bryophytes were tested for antibacterial and antifungal activity using standard Agar well diffusion assay according to Perez *et al.*, (1990). 5µg ciprofloxacin, 10 µg streptomycin and 200 µl of nystatine (5mg/ml) were used as positive controlfor the bacteria and fungi respectively. The assays were replicated twice and the mean values calculated (Sawant *et al.*, 2010; Ulka et al 2010).

Data management and analysis

Descriptive statistics were employed to present the phytochemical composition data. The findings of the antimicrobial activity were expressed as the mean \pm standard deviation (SD) from two separate replicates. To analyze the data, an analysis of variance (ANOVA) was performed, followed by the Duncan multiple tests to ascertain variations in the inhibition zones among different groups. Statistical significance was evaluated at a confidence level of P = 0.05.

3. RESULTS

Qualitative and Quantitative Phytochemical Analysis

The results of the qualitative phytochemical screening for the various fractions of F. *indica* are presented in Table 1. Alkaloids, flavonoids, and terpenoids were detected in all fractions. Saponins and tannins were found in every fraction except the n-hexane fraction. Tannins were absent in both the n-hexane and aqueous fractions but present in the others. Cardiac glycosides were exclusively present in the n-hexane and methanol fractions, with absence in the remaining fractions. Steroids were not identified in the n-hexane and aqueous fractions but were present in all other fractions. Phenols were only detected in the ethyl acetate and methanol fractions, while they were absent in the others. Anthraquinones were found in all fractions. Notably, all the phytochemicals tested were present in the methanol fraction of F. *indica*.

Table 2 provides a summary of the quantification results for the phytochemicals. The n-hexane fraction of *F. indica* contained 4% alkaloids, 16% flavonoids, and 20% terpenoids. The ethyl acetate fraction contained 4% alkaloids, 32% flavonoids, 3.50% saponins, no tannins, and 10% terpenoids. In the methanol fraction, there were 12% alkaloids, 32% flavonoids, 7.25% saponins, 1.10% tannins, and 8% terpenoids. The aqueous fraction comprised 4% alkaloids, 30% flavonoids, 4.55% saponins, 1.10% tannins, and 10% terpenoid

Antioxidant activity of the bryophyte

Table 3 provides a comprehensive overview of the DPPH free radical scavenging activity of the bryophyte. This table presents the absorbance-concentration relationship for all fractions of *F*. *indica* and compares it with the absorbance-concentration profile of ascorbic acid, a recognized standard antioxidant. Notably, all the fractions exhibited antioxidant capabilities. Among them, the n-hexane fraction displayed the highest activity, effectively scavenging 28.9% of DPPH at a concentration of $1000\mu g/ml$. In contrast, the ethyl acetate fraction exhibited the lowest activity, with a DPPH scavenging rate of 8.6% at the same concentration. The inverse correlation between absorbance and concentration signifies a higher percentage of scavenging as concentration increases, serving as an indicator of the extent of radical scavenging activity.

In Fig. 1, the Ferric ion Reducing Antioxidant Power (FRAP) of all *F. indica* fractions is graphically depicted. The elevation in absorbance can be attributed to the reduction of ferrous ions within the FRAP reagent. In general, antioxidant properties were observed across all fractions.

Fig. 2 presents the Total Antioxidant Capacity (TAC) content results for all fractions of *F*. *indica*. Notably, the ethyl acetate fraction of *F*. *indica* exhibited the highest TAC activity, registering a value of 2.834, while the lowest TAC (1.153) was observed in the methanol fraction at a concentration of 1000μ g/ml. The absorbance values directly correlate with the antioxidant activity and reducing power of the sample, with an increase in absorbance corresponding to an increase in Concentration.

	Fossombronia indica				
Test	Hexane	Ethyl acetate	Methanol	Aqueous	
Saponins	-	+	++	+	
Tannins	-	+	+	-	
Flavonoids	+	+	++	+	
Cardiac					
glycosides	+	-	+	-	
Anthraquinones	+	+	+	+	
Terpenoids	+	+	+	+	
Steroids	-	++	++	-	
Alkaloids	+	+	+	+	
Phenols	-	+	+	-	

Table 1: Qualitative phytochemicals detected in Fossombronia indica

+ represents Present, ++represents Abundantly present, - represents Absent

Table 2: Quantit	tative Phytochem	nical Composition	on of <i>Fossombronia indica</i>	\boldsymbol{x}

Name	Solvent	% Alkaloid	% Flavonoid	% Saponin	% Tannin	%Terpenoid
F. indica	Hexane	4.00	16.00	**	**	20.00
	Ethyl acetate	4.00	32.00	3.50	**	10.00
	Methanol	12.0	32.00	7.25	1.10	8.00
	Aqueous	4.00	30.00	4.55	1.17	10.00

** means Absent

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Conc.	Hexane	Ethyl acetate	Methanol	Aqueous
(µg/ml)	fraction F	fraction F	fraction F	fraction F
100	11.10	3.00	1.00	13.20
500	18.00	8.60	9.90	16.80
1000	28.90	8.60	12.80	18.40
a	a			

 Table 3: DPPH scavenging activity of Fossombronia indica

Conc. represents Concentration, F represents Fossombronia indica

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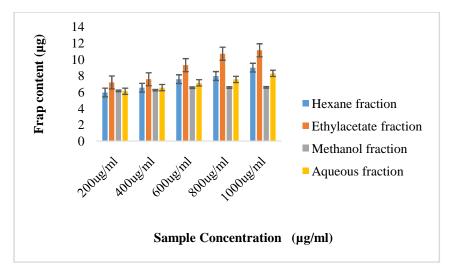


Figure 1: Ferric Ion Reducing Antioxidant Power of Fossombronia indica

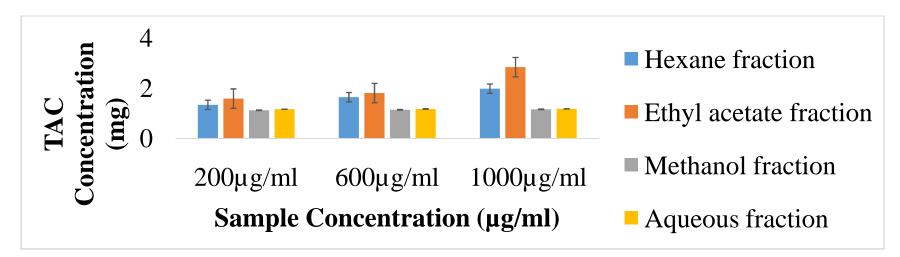


Figure 2: Total Antioxidant Activity (TAC) of the different fractions of Fossombronia indica relative to the standard

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Antimicrobial Susceptibility Assay

In Table 4, it was observed that *A. hydrophila* and *S. thyphi* displayed resistance to the n-hexane fraction of *F. indica*. On the other hand, *B. subtilis*, *P. aeruginosa*, and *S. aureus* exhibited sensitivity to all concentrations, while *E. coli* only showed sensitivity at the highest concentration. The ethyl acetate fraction, across all three concentration levels, demonstrated activity against the microorganisms, except for *E. coli* and *S. aureus*. Methanol fractions at all three concentration levels exhibited activity against the microorganisms, except for *A. coli* and *S. aureus*. Methanol fractions at all three concentration levels exhibited activity against the microorganisms, except for *A. hydrophila*. The lowest concentration of the aqueous fraction was ineffective against *B. subtilis*, whereas all other concentrations were active against the microorganisms. Notably, the ethyl acetate fraction displayed the highest level of activity, particularly against *P. aeruginosa* and *A. hydrophila*.

In Table 5, it was found that *C. albicans* displayed sensitivity to all fractions of n-hexane, ethyl acetate, methanol, and the aqueous fraction of *F. indica*. However, *A. niger* exhibited sensitivity exclusively to the ethyl acetate and methanol fractions of *F. indica*, while displaying resistance to the inhibitory effects of the n-hexane and aqueous fractions of *F. indica*.

	Conc.	Zone of inhibition (mm)					
Fractions	(mg/ml)	A. hydrophila	B. subtilis	E. coli	P. aeruginosa	S. typhi	S. aureus
N-Hexane	25	$0.00^{\mathrm{a}} \pm 0.00$	$15.00^{b} \pm 0.00$	$0.00^{\rm b}\pm0.00$	$12.50^b\pm0.71$	$0.00^{\mathrm{a}} \pm 0.00$	$11.00^{a} \pm 0.00$
	50	$0.00^{\mathrm{a}} \pm 0.00$	$15.50^{ab}\pm0.71$	$0.00^{\rm b}\pm0.00$	$15.50^{ab}\pm0.71$	$0.00^{\mathrm{a}} \pm 0.00$	$12.00^{a} \pm 1.41$
	100	$0.00^{a}\pm0.00$	$17.25^{a}\pm0.35$	$16.00^{a}\pm1.41$	$16.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$13.00^{a} \pm 1.41$
Ethyl acetate	25	$14.5^{b}\pm 0.71$	$10.00^{c} \pm 0.00$	$0.00^{\mathrm{a}} \pm 0.00$	$20.00^{c}\pm0.00$	$10.50^b\pm0.71$	$0.00^{\mathrm{a}}\pm0.00$
	50	$16.00^{b} \pm 0.00$	$12.00^b\pm0.00$	$0.00^{\mathrm{a}} \pm 0.00$	$22.00^b\pm0.00$	$12.00^{ab}\pm0.00$	$0.00^{\mathrm{a}} \pm 0.00$
	100	$21.00^{a} \pm 1.41$	$15.75^{a}\pm1.06$	$0.00^{a}\pm0.00$	$23.00^{a}\pm0.00$	$13.50^{a}\pm0.71$	$0.00^{\mathrm{a}}\pm0.00$
Methanol	25	$0.00^{\mathrm{a}}\pm0.00$	$12.50^{\rm c} \pm 0.71$	$11.00^b\pm0.00$	$11.00^{a} \pm 1.41$	$10.00^{\text{b}} \pm 1.00$	$10.25^b\pm0.35$
	50	$0.00^{\mathrm{a}} \pm 0.00$	$14.50^b\pm0.71$	$12.00^{ab}\pm0.00$	$13.00^{a}\pm0.00$	$11.00^b\pm0.00$	$11.50^b\pm0.71$
	100	$0.00^{a}\pm0.00$	$17.00^{a}\pm0.00$	$12.50^{a}\pm0.71$	$13.50^{a}\pm0.71$	$12.50^{a}\pm0.71$	$14.75^a\pm1.06$
Aqueous	25	$10.50^{a}\pm0.71$	$0.00^{c} \pm 0.00$	$9.00^{b} \pm 1.41$	$11.00^b\pm0.00$	$10.00^{\circ} \pm 0.00$	$10.00^{c} \pm 0.71$
	50	$12.00^{a}\pm0.00$	$10.00^b \pm 0.00$	$12.00^{a}\pm0.00$	$12.00^b\pm0.00$	$11.00^b\pm0.00$	$12.00^b\pm0.00$
	100	$12.00^{a} \pm 1.41$	$13.00^{a}\pm1.41$	$13.00^{a}\pm0.00$	$13.50^{a}\pm0.71$	$12.00^{a}\pm0.00$	$13.00^{a}\pm0.00$
Std. 1		$19.00^a\pm0.00$	$31.00^a\pm0.00$	$0.00^{\text{b}} \pm 0.00$	$0.00^{\text{b}} \pm 0.00$	$25.00^{a} \pm 1.41$	$34.50^{a} \pm 0.71$
Std. 2		$0.00^{b} \pm 0.00$	$\mathbf{28.50^b} \pm 0.71$	$18.00^{a}\pm1.41$	$29.00^{a}\pm0.00$	$25.00^{a}\pm0.00$	$25.00^{\text{b}} \pm 1.41$

Table 4: Antibacterial activity of Fossombronia indica

Std. 1 - Standard as Ciprofloxacin (5µg), Std. 2 – Standard as Streptomycin (10µg). Means with different superscripts differ significantly (p 0.05).

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Zone of inhibition (mm) F. indica Conc. A. niger C. albicans (mg/ml) Fractions N-Hexane 25 $0.00^{a} \pm 0.00$ $12.00^{\circ} \pm 0.00$ $14.00^{\rm b} \pm 0.00$ 50 $0.00^{a} \pm 0.00$ 100 $0.00^{a} \pm 0.00$ $15.00^a\pm0.00$ $10.50^{\rm b} \pm 0.71$ Ethyl acetate 25 $9.00^{\circ} \pm 0.00$ 50 $11.00^{b} \pm 0.00$ $12.00^{ab}\pm0.00$ 100 $12.50^a\pm0.71$ $13.50^{a} \pm 0.71$ $11.50^{b} \pm 0.71$ Methanol 25 $7.00^{\circ} \pm 0.00$ $12.50^{ab} \pm 0.71$ $10.00^b\pm0.00$ 50 100 $12.00^{a} \pm 0.00$ $14.00^{a} \pm 0.00$ $0.00^{a} \pm 0.00$ $12.00^{\circ} \pm 0.00$ 25 Aqueous $14.00^{b} \pm 0.00$ $0.00^{a} \pm 0.00$ 50 $0.00^{a} \pm 0.00$ $15.00^{a} \pm 0.00$ 100 $18.00^{b} \pm 0.00$ Std. 25 $12.00^{\circ} \pm 0.00$ $14.00^b\pm0.00$ $18.00^b\pm0.00$ 50 $15.50^{a} \pm 0.71$ $18.50^{\rm a}\pm0.00$ 100

Table 5: Antifungal activity of Fossombronia indica

Std. - Standard as Nystatine $(5\mu g)$. Means with different superscripts differ significantly (p 0.05).

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DISCUSSION

The presence of various phytochemical compounds, including saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, terpenoids, steroids, alkaloids, and phenols, was detected in nearly all fractions of t *F. indica*, indicating its rich phytochemical composition. These findings align with Adebiyi et al. (2012) study on mosses. Quantitative analysis further revealed that F. indica possesses high levels of alkaloids, flavonoids, terpenoids, and saponins, which are known for their medicinal properties such as antimicrobial, anti-malarial, anti-cancer, anti-inflammatory, and antioxidant effects, as reported by Stephen et al. (2009) and Guangyi et al. (2005). The presence of phenols in the bryophyte can potentially bind to proteins and reduce protein digestibility, which may lower the risk of heart diseases and certain cancers, aligning with the work of Mallikharjuna et al. (2007).

The various fractions of *F. indica* exhibited concentration-dependent scavenging effects, demonstrating high antioxidant potential in the three antioxidant assays conducted. This antioxidant activity can be attributed to the bryophyte's substantial content of flavonoids, terpenoids, tannins, saponins, and phenolic compounds, in agreement with the findings of Souvik et al. (2018). The ethyl acetate fraction of *F. indica* displayed the highest FRAP value (11.12), while the methanol fraction exhibited the lowest (6.58) at 1000μ g/ml, indicating increased antioxidant activity with rising concentration. However, it should be noted that the bryophyte's reductive capability is lower than that of the standard antioxidant compound, ascorbic acid. This is consistent with Greeshma and Murugan (2018) findings.

The Total Antioxidant Capacity (TAC) of the ethyl acetate fraction of the bryophyte displayed the highest content at the highest concentration, likely due to its rich phytochemical composition, the same trend also observed by Okafor et al. (2017). Similar patterns were reported in other bryophyte species such as *Atrichumundulatum*, *Polytrichumformosum*, and *Pleuroziumschreheri* by Chobot et al. (2006 and 2008). This aligns with the research on *Bryum moravicum* by Pejin et al. (2013) and *Thuidiumtamariscellum* by Mohandas and Kumaraswany (2018). Reinforcing the potential health-promoting effects of bryophytes in preventing degenerative diseases and ageing due to their volatile phenolic derivatives acting as antioxidants (Shaid, 1997).

The ethyl acetate fraction of *F. indica* exhibited the highest efficacy against *P. aeruginosa* at 100mg/ml, while the aqueous fraction showed the least effect against *E. coli* at 25mg/ml. This indicates that the bryophyte fractions possess bioactive components with antibacterial properties, as they inhibited the growth of these bacterial strains.

All the fractions of F. *indica* exhibited antifungal activity, except for the n-hexane and aqueous fractions, which did not inhibit the growth of *A. niger*. This suggests that the solvents effectively extracted the bioactive components responsible for this action. Notably, all fractions exhibited positive bioactivity against *C. albicans*, indicating the presence of antifungal substances in *F. indica*, comparable to the standard antifungal drug. These findings corroborate the report of Singh et al. (2006) on *Plagiochasmaappendiculatum* antifungal activity and Milen et al. (2008) findings regarding *Pleuroziumschreberi* antifungal activity against various pathogenic fungi, *including A. niger, A. flavus, A. ochraceus, A. versicolor, Trichoderma viridae*, and *C. albicans*.

CONCLUSION

This study highlighted the abundance of bioactive compounds within *F. indica*, which exhibit antimicrobial properties and act as abundant reservoirs of antioxidants to combat oxidative damage. Consequently, it is recommended as a promising material for drug development.

DECLARATION OF INTEREST

The authors alone are responsible for the writing and content of the paper with no competing interest

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