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Phytocompounds of *Bistorta macrophylla* (D. Don) Sojak. as bioavailability enhancers of fluconazole and amphotericin B to better manage *Candida species* infections

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Abstract

Bistorta macrophylla (D. Don) Sojak. is a medicinal plant of high altitude and so far, not been scientifically explored? Since prehistoric times, *B. macrophylla* has been used to cure stomach pain, pyretic fever, flu, lungs infections, diarrhea, vomiting. The present research was aimed to examine the phytochemicals, antifungal, and synergistic potential of methanolic extracts of *B. macrophylla.* Methanolic extract of *B. macrophylla* was found to have high phenolic (191.18 ± 29.18 mg g^{-1} GAE) and flavonoid (26.71 ± 3.21 mg g^{-1} RE) content. Methanolic extract also demonstrate strong antifungal action with diameter of zone of inhibition of 17.5±0.5 mm (fungicidal) against both the strains of *C. albicans* (MTCC277 and ATCC90028). The minimal inhibitory concentration (MIC) of methanolic extract was found to be 62.5 µg ml^{-1} against *C. albicans* (MTCC277 and ATCC90028). In addition, the combination of methanolic extract of *B. macrophylla* with antifungal antibiotics (fluconazole and amphotericin B) showed synergistic interaction with MIC reduction from 4-128 folds against both candida strains. GC-MS analysis of methanolic extract revealed the presence of 15 major phytocompounds with area more than 1%. Molecular docking showed that sucrose and 9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione has highest binding energy of -6.3 and -5.1 KJ/mol against Cytochrome P450 14 alpha-sterol Demethylase (PDB ID: 1EA1) protein respectively. Combination of methanolic extract of *B. macrophylla* with antifungal antibiotics (fluconazole, amphotericin B) can be used to treat drug-resistant candida.

1. Introduction

Since prehistoric time, medicinal plants and herbs have been discovered and used in folk medicine (Stojanoski 1999). Now phytocompounds are at the forefront of drug discovery. According to World Health Organization (WHO), “Any plant whose one or more part, contain substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs” is classified as medicinal plant. WHO has estimated that about 80% of the World population rely almost exclusively on traditional medicine for their primary healthcare needs (Farnsworth 1988). Medicinal plants are the “backbone” of traditional medicine system, and most of the people in the less developed countries utilized these traditional medicinal plants on a regular basis (Davidson-Hunt 2000) for their well-being. Plants produce or secrete an array of chemical compounds and use them for various biological functions including defense against insects, fungi and bacteria. Scientists are now exploring these phytocompounds as novel therapeutics approach to solve the emerging issues of multi-drug resistance (Rolta et al. 2020b; Rolta et al. 2021; Salaria et al. 2021). Antimicrobial resistance is a major threat to animal and human health development, affecting our ability to treat a range of infections. Treatments for a growing number of infections have become less effective in many parts of the world due to emergence of drug resistance (WHO 2016). Among many other diseases, candidiasis caused by *Candida albicans* is one of the major concerns (López-Martínez 2010). Fluconazole and amphotericin B are the most commonly and widely used antibiotics to treat Candidiasis. Some strains of *Candia* have developed resistance (antifungal resistance) to these antibiotics and are difficult to treat (Helmerhorst et al. 1999). The rise of antifungal resistance is very high and discovery of new antibiotics is very slow. Centres for disease control and prevention (CDC) has reported that some strains of *Candida* are highly resistant to even first line and second line of antifungal medications, such as fluconazole and the echinocandins (anidulafungin, caspofungin, and micafungin). *Candida auris* has been reported to be resistant to fluconazole and amphotericin B and now it has become a serious health concern (Satoh et al. 2009; Lockhart et al. 2016). The individual with drug resistant infection has to consume higher dosage of antibiotics, which in turn leads to emergence of drug resistance and also

responsible for many side effects and ultimately life expectancy (Gould et al. 2013). The mechanisms of antibiotic resistance in mainly due to mutation in gene encoading the target site of FQs (DNA gyrase and topoisomerase IV), over expression of efflux pumps and protection of the FQ target site by a protein designated Qnr (Munita and Arias 2016). The combination therapy of two or more antibiotics (synergism) has been in practice for some, but this has also resulted in selection pressure on pathogens and drug toxicity (Dudhatra et al. 2012).

Therefore, the current need is to search for molecules that are not antibiotics itself, but enhance the potency /availability of the antibiotics. Such molecules should be non-toxic and enhance the bioavailability of antibiotics in combination and act synergistically. Potent bio-enhancers could potentially lower the dosage of antibiotics and therefore reduced toxicity to the host. Phytochemicals of medicinal plants have huge potential to be developed as bio-enhancer of antibiotics and feed the drug discovery pipeline to develop new therapies to manage candidiasis. India has huge diversity of medicinal plants and rich system of traditional medicines and ethno pharmacology. Especially, Himalayan region houses 1748 species of unique medicinal plants with various traditional and modern therapeutic uses (Samant et al. 1998), including 675 species of wild edible plants (Samant and Dhar 1997) and 118 species of medicinal plants yielding essential oils. High altitude of Himalayas is a unique habitat that resulted in the accumulation of unique phytoconstituents in medicinal plants and warrants their exploitation in a holistic manner to counter the problem of multidrug resistance and for the well-being of people. The Indian sub-continent is suitable for cultivation of large number of medicinal and aromatic plants, which can be used as raw materials for pharmaceuticals, perfumery, cosmetics, flavors and food. The Chanshal valley of Himachal Pradesh is regarded as hub of medicinal plants and trees. It contains several species of medicinal plants and trees e.g. *Rheum emodi*, *Juniperus communis*, *Bistorta macrophylla*, *Jurinea macrocephala, Picrorhiza kurroa, Pleurospermum bruninis* and *Betula utilis* etc. and people of Dodra Quar and Rohru region utilize these medicinal plants or trees for the cure and preventions of different diseases (Jaundice, wound healing, fever, cough, respiratory problems, boils, nerve disorder, dysentery ulcers etc.) since ancient time.

B. macrophylla is one of the important and unexplored medicinal plant species of alpine region of Himalayas and is commonly known as Kukhri, Chhotaninayin, Kande-re-ninai in Hindi and Snakeweed in English. *B. macrophylla* belongs to family polygonaceae. *B. macrophylla* is a stout perennial herb and arise from a woody root stock (rhizome), reticulate. It is native to mountain regions of West-China, Pakistan, Bhutan, North India (Himachal Pradesh, Uttarakhand), West India (Western Ghats) and Nepal (Chauhan 1999). In India, it is distributed at altitudes ranging from 3300 to 3800 meters height in the temperate and subtropical regions of Himalayas (Chauhan 1999). Rhizomes of *B. macrophylla* are widely used in Ayurvedic and traditional medicine as antidiarrheal, antidysenteric, alleviates stomach pain, anti-inflammatory, and anti-pyretic (Gaur 1999). Traditionally, *B. macrophylla* is used to cure the stomach problems and paste of *B. macrophylla* rhizome is given to children and infants for stomach problems. Older patients chew the roots for the same problem (Chauhan 1999). Paste and powder of *B. macrophylla* roots was taken orally to cure fever, ulcer and toothache. Paste of *B. macrophylla* roots by prepared by rubbing roots on hard surface of stone with few drops water. It is also used to cure tuberculosis, inflammation, pyretic fever, flu, lungs disorders, diarrhea, vomiting, arthritis, gout, kidney stones or hyperacidity and hypertension (Wangchuk et al. 2016). *B. macrophylla* is known for its traditional medicinal values, but not explored scientifically, except one report by Chandra et al. (2016). Therefore, the current study was designed to study the potential of methanolic extract of rhizome of unexplored

B. macrophylla as bioavailability enhancer of fluconazole and amphotericin B against to manage candidiasis and identify the major phytocompounds by GC-MS analysis.

2. Materials And Methods

2.1 Collection of *B. macrophylla* samples and preparation of rhizome extract using Soxhlet extraction method

The rhizomes of *B. macrophylla* were collected from the Chanshal valley of District Shimla (latitude-31.196925 ºN and longitude: 77.988675 ºE), Himachal Pradesh (India) in the month of August, 2017 (Figure 1). The collected plant samples of *Bistorta macrophylla* (D. Don) Soják were identified from Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh (India) with reference no. UHF-13583 and plant name has been verified at http://www.theplantlist.org.

The rhizome of *B. macrophylla* was thoroughly washed with running tap water to remove the soil particles and surface sterilized using 70% ethanol for 2 minutes, followed by washing with sterilized distilled water. The rhizomes were cut into small pieces and dried in hot air oven at 40 ºC (until no further reduction in weight for 6 h) and then ground to fine powder with the help of electric grinder.

About 50 grams of dried powder of rhizome of *B. macrophylla* was subjected to methanol (500 ml) extraction using hot continuous method in a Soxhlet apparatus. The extract was filtered through Whatmann filter paper no. 1 and the collected filtrate was dried at 40 ºC. The dried methanolic extract was stored at 4 ºC in air tight bottles until further use.

2.2 Qualitative analysis of phytochemicals present in methanolic extract of *B. macrophylla*

The rhizome extract of *B. macrophylla* was tested for the presence of major phytocompounds such as phenolics, flavonoids, tannins, saponins, alkaloids, glycosides, phytosteroids and carbohydrate by protocols described earlier (Khandelwal 2008). For the detection of alkaloids and glycosides, 50 mg of methanolic extract was dissolved in 5 ml of diluted HCl (1%, w/w) and filtered through Whatman filter no1. The resultant filtrate was used for the detection of alkaloids and glycosides. On the other hand, 50 mg of methanolic extract was dissolved in 5 ml of sterilized water and then filtered. The filtrate was used for the detection of phenolics, tannins, phytosterols, phytosteroids, carbohydrate, flavonoids, proteins and amino acids.

2.3 Quantitative analysis of phenolic contents and flavonoids in methanolic extract of *B. macrophylla* rhizome

2.3.1 Quantification of total phenolic contents

The total phenolic content of methanolic extract of *B. macrophylla* rhizome was determined by using Folin-ciocalteau reagent (Singleton 1999) total phenolic content was calculated from the calibration curve of gallic acid (5-100 µg ml^{-1}) and expressed in terms of gallic acid equivalents (GAE) per gram of the extract and calculated using the following equation:

$$C = \frac{c \times V}{m}$$

Where ‘C’ is total content of phenolic compounds in mg g-1 plant extract in GAE, ‘c’ is the concentration of Gallic acid estimated from the calibration curve (mg ml-1), ‘V’ is the volume of extract in milliliter and “m” is the weight of crude plant extract in grams.

2.3.2 Quantification of total flavonoid content

The total flavonoid content of ethanolic extract of rhizome of *B. macrophylla* was quantified using aluminium chloride (AlCl3) method (Zhishen et al. 1999). The flavonoid content was calculated from standard curve of rutin (5- 100 µg ml^{-1}) and expressed as rutin equivalents (RE) per gram of the extract and was calculated by the following equation:

$$C = \frac{c \times V}{m}$$

where ‘C’ is total content of flavonoid compounds in mg g-1 plant extract in rutin equivalent; ‘c’ is the concentration of rutin calculated from the calibration curve in mg ml^{-1}, ‘V’ is the volume of extract in ml, and ‘m’ is the weight of crude plant extract in grams.

2.4 Analysis of antifungal activity in methanolic extract of *B. macrophylla* rhizome

2.4.1 Microbial Strains and culture conditions

Two fungal strains such as *Candida albicans* (ATCC90028 and MTCC277) were used to test the antifungal activity of methanolic extract of *B. macrophylla* rhizome. Both the fungal strains were obtained from Yeast Biology Lab, Shoolini University, Solan, Himachal Pradesh, India. The fungal strains were grown in yeast extract, peptone and dextrose (YPD) broth at 30 ºC with shaking at 200 rpm. The cell turbidity of the culture was adjusted by comparing with 0.5 McFarland standards (Lahuerta and Pérez-Gracia 2012), which is approximately equivalent to 2×10^8 colony forming units (CFU) ml^{-1} of the culture for antifungal assay. The culture media used in this study were purchased from Himedia Biosciences, Mumbai (India).

2.4.2 Agar well diffusion and Broth dilution assays for measuring antifungal activity of methanolic extract of *B. macrophylla* rhizome

Antifungal activity was tested by using agar well diffusion method (Perez 1990; Rolta et al. 2018 a, b) About 25 ml of YPD agar medium was poured in sterile 100 mm petri-dish and allowed to solidify. Then, fungal culture of optical density of 0.12~0.15 at 530 nm equivalent to 0.5 McFarland standard was uniformly spread on the surface of the YPD agar medium using sterile cotton swabs and plated were allowed to dry under aseptic conditions. The wells were punched with the cork borer (6 mm) in the agar and 50 µg (1 µg ml^{-1}) rhizome extract was loaded in the wells and allowed to diffuse. Assay plates were incubated of 48 h at 30 ºC and the zone of inhibition was measured using Hi Antibiotic Zone scale-C (Himedia Biosciences, Mumbai (India). Fluconazole filter disk (10 µg) purchased from Himedia Biosciences, Mumbai (India) was used as a positive

control and dimethyl sulphoxide (DMSO) and methanol alone were used as solvent alone as a control in the antifungal assay. The tests were performed in triplicate and results were recorded as mean ± SD.

2.4.3 Broth dilution assay to determine the minimum inhibitory concentration (MIC) of methanolic extract of *B. macrophylla* rhizome

The minimum inhibitory concentration (MIC) of the methanolic extract of rhizome of *B. macrophylla* was measured by broth dilution method described under CLSI guidelines using 5-tripheny tetrazolium chloride (CLSI 2012). The methanolic extract was dissolved in DMSO and geometric dilutions ranging from 500–0.025 µg ml^{-1} were prepared in a 96-well microtiter plate, including one growth control (YPD broth containing DMSO) and a positive control (YPD broth inoculated with fungal culture and containing amphotericin B (25 µg) or fluconazole (25 µg). Assay plates were incubated for 48 hours at 30 ºC. Following incubation, 5-tripheny tetrazolium chloride (5 µg) was added to each well and incubation was continued for another 2 h. Change in colour from purple to pink or colourless was observed and used as a measure to calculate the MIC. The lowest concentration at which colour change appeared was considered as the MIC value as descried previously (Rolta et al. 2018a, Rolta et al. 2020a, b).

2.4.4 Synergistic enhancement of antifungal activity of fluconazole and amphotericin B against fungal strains when combined with methanolic extract of rhizome of *B. macrophylla* using broth dilution method

Synergistic interaction of phytocompounds present in methanolic extracts of *B. macrophylla* and fluconazole or amphotericin B were determined using checkerboard method (van Vuuren et al. 2011; Eumkeb et al. 2012, Dev et al. 2017; Rolta et al. 2018b; Bonapace et al. 2020). Briefly, the different combinations of methanolic extract of *B. macrophylla* and antifungal antibiotics were prepared and broth dilution method was performed to determine the MIC as shown in the schematic diagram (Figure 2). The checkerboard method is often combined with calculation of fractional inhibitory concentration (FIC) index (FICI). The FIC was derived from the lowest concentration of antibiotic and plant extracts combination showing no color change. FIC value for each compound was calculated using the following formula:

FICI = FIC (fluconazole or amphotericin B) + FIC (rhizome extract of *B. macrophylla*)

where, FIC (fluconazole or amphotericin B) = MIC of antibiotic in combination/ MIC of antibiotic alone; FIC (rhizome extract of *B. macrophylla*) = MIC of rhizome extract of *B. macrophylla* in combination/ MIC of rhizome extract of *B. macrophylla* alone

The interactions were classified as being synergistic with ΣFIC values of ≤0.5, additive (≥ 0.5–1.0), indifferent (≥1.0–≤4.0) or antagonistic (ΣFIC > 4.0) (Nidhi et al. 2020; Rolta et al. 2020 a,b; Salaria et al. 2021; Mehta et al. 2021)

2.5 GC-MS of methanolic extract of *Bistorta macrophylla* rhizome

To identify the phytocompounds in methanolic extract of *Bistorta macrophylla*, methanolic extract was analysis using GC-MS (Thermo Trace 1300 GC coupled with Thermo TSQ 800 Triple Quadrupole MS) fitted with a BP 5MS capillary column (30 m x 0.25 mm, 0.25 µm film thickness). Injector temperature was 250 ºC. The

column temperature was programmed from 110 ºC to 200 ºC, temperatures held for 110 ºC for 2 minutes, gradually increased to 200 ºC at 10 ºC minutes-1 and then 280 ºC at 5 ºC minutes^{-1} kept there for 9 minutes. Helium was used as a carrier gas, at a flow rate of 1.0 ml minutes^{-1} and mass spectra were recorded in the scan mode. The ionization voltage was 70 eV. The split ratio was 10:1. The ion source temperature was 230 ºC, Interface temperature was 280 ºC. Solvent cut time was 3 minutes. For the analysis, 1 µl of 1mg ml^{-1} of the sample was used. The constituents of extract were identified based on their retention time (Rt) with respect to the reference. The scan range was 45-450 m/z. The identification of compounds was based on matching unknown peaks with MS-data bank (NIST 2.0 electronic Library).

2.6 Molecular docking of *B. macrophylla* compounds with target protein of *C. albicans*

2.6.1 Bioinformatics tools: AutoDock Vina (Trott and Olson, 2010) Open Babel GUI (O'Boyle et al. 2011), Molispiration (https://www.molinspiration.com/), PROTOX-II (http://tox.charite.de/protox_II/) and discovery studio and Chimera 1.8.1 were used in this study.

2.6.2 Retrieval and preparation of target protein

3-dimentional structure of *C. albicans* target protein (Cytochrome P450 14 alpha-sterol Demethylase PDB ID: 1EA1) (Podust et al. 2011) were retrieved from protein data bank (https://www.rcsb.org/). Active site was predicted on the basis of previous studies (Rolta et al. 2020C and Salaria et al. 2021).

2.6.3 Ligand preparation

Three dimensional structures of *B. macrophylla* compounds such as 1-(3-Hydroxypropyl)-2-piperidinone, 1,3-Dioxolane, 5-Keto-D-fructose, 9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione, Benzoic acid, 2-(dimethylamino) ethyl ester, Benzoic acid, 2-(dimethylamino) ethyl ester, N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane, Sucrose and standard antifungal antibiotic (fluconazole) were retrieved from Pubchem (www.pubchem.com) in .sdf format. Ligand energy was minimized by using chemdraw 3D 15.0 and ligand was prepared by using autodock tool 1.5.6.

2.6.4 Molecular docking of *B. macrophylla* compounds with *C. albicans* target

All the selected ligands (1-(3-Hydroxypropyl)-2-piperidinone, 1,3-Dioxolane, 5-Keto-D-fructose, 9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione, Benzoic acid, 2-(dimethylamino) ethyl ester, Benzoic acid, 2-(dimethylamino) ethyl ester, N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane, Sucrose) were used to perform molecular docking studies with fungal proteins (1EA1) to find the potential drug. In the current research molecular docking was performed by using AutoDock vina (Trott and Olson 2010).

2.7 Drug likeness calculations of *B. macrophylla* compounds

The purpose of the drug scan was to see if certain phytochemicals met the drug-likeness criteria. For assessing drug similarity properties such as amount of hydrogen acceptors, Lipinski's filters were used. Drug likeness prediction was done by using online server Molinspiration (http://www.molinspiration.com) were applied for examining drug likeness attributes as including quantity of hydrogen acceptors (should not be more than 10),

quantity of hydrogen donors (should not be more than 5), molecular weight (mass should be more than 500 daltons) and partition coefficient log P (should not be less than 5). The smiles format of each of the phytochemical was uploaded for the analysis (Rosell and Crino 2002).

2.8 ADME/T screening and toxicity prediction of *B. macrophylla* compounds

ADME/T screening was done to determine the absorption, toxicity, and drug-likeness properties of selected phytocompounds. Canonical SMILES of *B. macrophylla* phytocompounds (1-(3-Hydroxypropyl)-2-piperidinone, 1,3-Dioxolane, 5-Keto-D-fructose, 9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione, Benzoic acid, 2-(dimethylamino) ethyl ester, Benzoic acid, 2-(dimethylamino) ethyl ester, N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane, Sucrose) uploaded on PROTOX-II webservers (Charite University of Medicine, Institute for Physiology, Structural Bioinformatics Group, Berlin, Germany). PROTOX is a Rodent oral toxicity server predicting LD50 value and toxicity class of query molecule. The toxicity classes are as follows: (i) Class 1: fatal if swallowed (LD50 ≤5), (ii) Class 2: fatal if swallowed (55000) (Banerjee et al. 2018).

3. Result And Discussions

3.1 Qualitative and quantitative estimation of phytocompounds in methanolic extract of *B. macrophylla* rhizome

3.1.1 Qualitative analysis of phytochemicals

Methanolic extract of rhizome of *B. macrophylla* was subjected to phytochemical screening such as alkaloids, flavonoids, phenolic compounds and tannins, carbohydrates, glycosides, steroids, saponins, proteins and amino acids. The qualitative screening revealed the presence of phytocompounds such as phenolics, tannins, flavonoids, carbohydrates, alkaloids and phytosteroids. However, glycosides, terpenoids and saponin were undetectable. Summary of phytochemical screening is shown in Table 1. Previously, Chandra et al. (2016) have also investigated the phytochemicals in various extracts of whole plant of *B. macrophylla* and reported that alkaloids and phytosteriods were undetectable in methanolic as well as in water extracts. Similar to our study, Chandra et al. (2016) showed the presence of phenolics, tannins, flavonoids, carbohydrates, glycosides, and saponins in methanolic and water extracts. Petroleum ether extract showed the presence of phenolics and tannins only. This variation in phytoconstituents may be possibly due to: 1) difference in geographical locations of the sample collection; sample collection at different time of the year; 3) different genotypes. The results of current study and Chandra et al. (2016) indicate that accumulation of phytocompounds could be affected by geographical location, month of sample collection along with genotype. Nayeem et al. (2017) reported the seasonal and geographic variation in phytoconstituents of *Tribules terrestris collected from Rajha*, Northern Border province Saudi Arabia and Bangalore, Karnataka, India in summer and winter. Study from Inbathamizh and padmini (2013), suggested the effect of geographical distribution on phytochemical and antioxidant activity of *Moringa oleifera* flowers.

Table 1
Summary of phytocompounds present in methanolic extract of rhizome of *B. macrophylla.*

Phytoconstituents	Tests	Methanolic extract
Phenolics and tannin	Ferric chloride test	+
	Gelatin test	+
Flavonoids	Lead acetate test	+
Carbohydrates	Fehling solution test	+
Glycosides	Borntrager test	-
Alkaloids	Dragendorff test	+
Saponin	Foam test	-
Terpenoids	Salkowsky test	-

'+' indicates the presence and '' indicates the absence of phytochemicals. The intensity of the colour produced was taken as an indication of amount of particular phytochemicals present.

3.1.2 Methanolic extract of *Bistorta macrophylla* rhizome is rich in phenolic compounds

Total phenolic contents (TPC) were determined using Folin-ciocalteau method (Singleton 1999) and the values were expressed as GAE, whereas, total flavonoid content (TFC) was measured by aluminium chloride method (Zhisen et al. 1999) and the values were expressed in terms of RE (rutin equivalent). *Bistorta macrophylla* rhizome extract was rich in total phenolic content (191.18±29.18mg g^{-1} GAE) as compared to flavonoid content (26.71±3.21 mg g^{-1} RE). Our data is in contrast to previous report by Chandra et al. (2016), where they showed that total phenolic and flavonoid content of methanolic extract of *Bistorta macrophylla* were 76.14 mg g^{-1} GAE and 51.55 mg g^{-1} RE, respectively. Our study showed that phenolic content was almost 2.5 times more, but flavonoid content was half as compared to reports by Chandra et al. (2016). Study from Rosendal et al. (2020) is the evidence of variation in phenolics and flavonoids due to different geographical regions.

3.2 Analysis of antifungal activity of methanolic extract of *B. macrophylla* rhizome by agar well diffusion and broth dilution method

An antifungal activity assay was performed by using agar well diffusion method and observed as zone of inhibition against the tested fungal strains. Methanolic extract of *B. macrophylla* showed inhibition to growth of fungal strains. Antifungal activity was fungicidal (cell death) against *C. albicans* (MTCC27 and ATCC90028); whereas fluconazole and amphotericin B were fungi static (growth arrest) against *C. albicans* (MTCC277 and ATCC90028) shown in Table 2 and figure 3a.

Table 2
Qualitative and quantitative measurement of antifungal activity of methanolic extract of rhizome of *B. macrophylla* using agar well diffusion and MIC assays.

Fungal strains	Zone of inhibition (ZOI) in mm			Minimum inhibitory concentration (MIC) in µg/ml		
	Methanolic Extract	Fluconazole	Amphotericin B	Methanolic Extract	Fluconazole	Amphotericin B
C. albicans (MTCC277)	17.5 (**)	17.5 (*)	12 (*)	62.5	31.25	31.25
C. albicans (ATCC90028)	17.5 (**)	17.5 (*)	12 (*)	62.5	15.6	31.25

Double star (**) indicates fungicidal activity (cell death); whereas single star (*) indicates fungistatic activity (growth arrest). Antifungal activity was measured as zone of inhibition (mm) and minimum inhibitory concentration (MIC).

MIC of methanolic extract of *B. macrophylla* was 62.5 µg ml^{-1} against both fungal strains *C. albicans* (MTCC277 and ATCC 90028). On the other hand, the MIC of fluconazole was found 31.25 µg ml^{-1} against *C. albicans* (MTCC277), and 15.6 µg ml^{-1} *C. albicans* (ATCC 90028). The MIC of amphotericin B was 31.25 µg ml^{-1} against both strains of *C. albicans* (MTCC277 and ATCC 90028) (Table 2 and Figure 3b). *B. macrophylla* rhizomes have been reported for wound healing by local healers of higher altitudes of Uttarakhand, India (Phondani 2011). Also, rhizome and leaves of *Bistorta affinis* and *Bistorta amplexicaulis* are known for the treatment of skin irritation, dermatitis, eczema, measles (Rahman et al. 2018).

3.3 Synergistic enhancement of antifungal activity of fluconazole and amphotericin B against fungal strains

Methanolic extract of rhizome of *B. macrophylla* showed synergistic potential with fluconazole and amphotericin B against fungal strains. To analyze the synergistic potential between antifungal antibiotics (fluconazole and amphotericin B) and methanolic extract of rhizome of *B. macrophylla*, nine combinations based on MIC of antibiotics and methanolic extract of rhizome of *B. macrophylla* were prepared. Based on the minimum FICI value, the combination of extract equivalent to MIC (62.5 µg ml^{-1}) and fluconazole equivalent to ½MIC (15.625 µg ml^{-1}) and ½ MIC of extract (31.25 µg ml^{-1}) and ½ MIC of amphotericin B (15.625 µg ml^{-1}) showed best synergistic potential against *C. albicans* (MTCC277). The synergistic combination of methanolic extract and fluconazole enhanced the potency of the fluconazole and methanolic extract by 32 folds against *C. albicans* (MTCC277). The combination of amphotericin B with methanolic extract enhanced the potency of amphotericin B and methanolic extract by 128 folds. Combination of ½ MIC of extract (31.25 µg ml^{-1}) and MIC of fluconazole (15.625 µg ml^{-1}); ½ MIC of extract (31.25 µg ml^{-1}) and ½ MIC of amphotericin B (15.625 µg ml^{-1}) showed best combinations against *C. albicans* (ATCC90028) as shown in Table 3. The synergistic potential of fluconazole in combination with methanolic extract was enhanced by 116 folds and 16 folds for

methanolic extract. On the other hand, the synergistic potential of amphotericin B in combination with methanolic extract was enhanced by 128 folds for amphotericin B and methanolic extract.

Table 3
FIC index showing synergistic potential of methanolic extract of *B. macrophylla* against *S. cerevisiae* (H1086), *C. albicans* (MTCC277), *C. albicans* (ATCC90028) with fluconazole and amphotericin B.

ME	Antibiotic	Fractional Inhibitory Concentration Index (FICI)							
		C. albicans (MTCC277)				*C. albicans* (ATCC90028)			
		Ab1	Fold	Ab2	Fold	Ab1	Fold	Ab2	Fold
2MIC	2MIC	0.024	ME-16 Ab-16	0.031	ME-32 Ab-32	0.063	ME-16 Ab-16	0.007	ME-64 Ab-64
2MIC	MIC	0.018	ME-8 Ab-16	0.032	ME-32 Ab-32	0.032	ME-32 Ab-32	0.031	ME-32 Ab-32
2MIC	MIC/2	0.015	ME-16 Ab-16	0.016	ME-32 Ab-32	0.016	ME-16 Ab-16	0.016	ME-32 Ab-32
MIC	2MIC	0.013	ME-16 Ab-16	0.063	ME-32 Ab-32	0.062	ME-16 Ab-16	0.001	ME-32 Ab-32
MIC	MIC	0.001	ME-16 Ab-16	0.031	ME-32 Ab-32	0.062	ME-8 Ab-8	0.31	ME-32 Ab-32
MIC	MIC/2	0.004	ME-32 Ab-32	0.015	ME-32 Ab-32	0.050	ME-16 Ab-16	0.015	ME-32 Ab-32
MIC/2	2MIC	0.251	ME-16 Ab-16	0.003	ME-32 Ab-32	0.063	ME-16 Ab-16	0.008	ME-32 Ab-32
MIC/2	MIC	0.252	ME-8 Ab-8	0.032	ME-32 Ab-32	0.031	ME-16 Ab-16	0.032	ME-32 Ab-32
MIC/2	MIC/2	0.013	ME-8 Ab-8	0.0165	ME-128 Ab-128	0.007	ME-32 Ab-32	0.017	ME-128 Ab-128

ME- Methanolic extract, Ab1- Fluconazole, Ab2 Amphotericin B. Red shades indicates the best combination of extract and antibiotic

So far, there is no study on the synergistic potential of *B. macrophylla* extract in the literature against fungal strains. This is the first study to show the antifungal activity of *B. macrophylla* extract. In addition to antifungal activity, methanolic extract of *B. macrophylla* also acts synergistically with antifungal antibiotics (fluconazole and amphotericin B) and increases the potency. The Indian patent application for this study has been submitted (Kashyap et al. 2018)

Synergistic activity of plant extract and antibiotics were reported in literature and such combinatorial formulations can be considered an alternative to antibiotics for the treatment of multi drug resistant bacterial and fungal pathogens (Yu et al. 2005; Al-Bayati, 2008; Rolta et al. 2018 a,b; Nidhi et al. 2020; Rolta et al. 2020a). Rolta et al. (2020b) reported the synergistic activity of emodin extracted from chloroform fraction of *Rheum emodi* rhizome extract (methanolic) in combination with antibacterial and antifungal antibiotics. Emodin increases the efficacy of antibiotics 4-257 folds against bacterial and fungal pathogens. Mehta et al. 2021 reported the synergistic activity of ethyl-acetate extracts of *P. integerrima*, *O. sanctum*, *C. asiatica*, *M. charantia*, *Z. officinale*, and *W. somnifera* in combination with ciprofloxacin and tetracycline against *Salmonella typhimurium.* Salaria et al. 2021 reported the synergistic activity of *Thymus serpyllum* essential oil and thymol against *E. coli, S. aureus and K. pneumoniae* in combination with tetracycline and vancomycin; they also reported the synergistic activity against *C. albicans* (MTCC277 and ATCC 90028) in combination with Fluconazole and amphotericin B by using checkerboard method.

3.5 GC-MS of methanolic extract of *Bistorta macrophylla* rhizome

GC-MS is important technique for the quality evaluation of natural products Yi et al (2017). GC-MS analysis of the methanolic extract of *B. macrophylla* showed the presence of 15 major with peaks with area (%) corresponding to more than 1. These phytocompounds were identified by comparing the mass spectra of the constituents with the NIST mass spectral library (https://chemdata.nist.gov/) and are summarized in Table 4. The list of phytocompounds identified in the methanolic extract of *B. macrophylla* is summarized in Table 4. The profile of mass spectra showing the retention time (RT) and relative abundance of all the phytochemicals identified in the methanolic extract of *B. macrophylla* is shown in Figure 4. The present study is the first report on GC-MS analysis of methanolic rhizome extract of rhizome of *B. macrophylla.* GC-MS peaks showing more than 1% relative abundance were analyzed and reported. Among all the phytocompounds, sucrose (41.07%), benzoic acid, 2-(dimethylamino) ethyl ester (18.24%), 9, 9-Dimethoxybicyclo [3.3.1] nona-2, 4-dione (7.11%) were major constituents. The phytocompounds such as 5-Keto-D-fructose (3.25%) and sucrose (41.07%) have been reported as sweetener agent (Herweg et al. 2018; Tappy et al. 2010) and antidiabetic properties (Wyrobnik and Wyrobnik 2018) Benzoic acid (18.24%) is known for its antibacterial activity (Park et al. 2001) against *Staphylococcus aureus* ATCC 6538P, and *Pseudomonas aeruginosa* ATCC 15522. α-D-Glucopyranoside have reported for its cardioprotective, neuroprotective, antidiabetic and anti-osteoporotic activity (Altameme et al. 2015). Similarly, Ko et al. (2018) reported that linoleic acid, ethyl ester as antimicrobial and anti-inflammatory agents, mention few lines about data, which microbes etc. Decanoic acid (capric acid) is a class of medium chain fatty (MCFA) that are known to create pores in the membranes and impair the cell permeability (Choi et al. 2013) and leakage of the cytoplasmic content, and eventual cell death (Tsuchido et al). 1, 3-Dioxolane derivatives have been reported for antibacterial and antifungal activity against *S. aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and against a yeast *Candida albicans* ATCC 10231 (Küçük et al. 2011). Similarly, antimicrobial activity of organic–inorganic hybrid films based on gelatin and organomodified silicones (Heptamethyltrisiloxane) have been reported by Wojciechowska et al. (2018). Either alone or in combination, these phytocompounds present in the rhizome extract of *B. macrophylla* could be responsible for antifungal activity, antioxidant activity, and enhancing the potency of fluconazole and amphotericin B.

Table 4
Summary of compounds indentified in methanolic extract of *B. macrophylla* through GC-MS analysis.

Compound name/CID	RT (min)/Area %	Molecular formula	Pharmacological properties
5-Keto-D-fructose /99513	3.40 /3.25	$C_6H_{12}O_6$	Sweetener agent and antidiabetic (Herweg et al. 2018; Wyrobnik and Wyrobnik 2018)
N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane /588924	4.49 /1.48	$C_6H_{12}N_2S$	No activity reported
1-(3-Hydroxypropyl)-2-piperidinone/574874	5.34 /0.77	$C_4H_4N_4O_3$	No activity reported
α-D-Glucopyranoside/95143	5.88 /1.43	$C_{18}H_{32}O_{16}$	Cardioprotective, neuroprotective, antidiabetic and anti-osteoporotic activities (Altameme et al., 2015).
Cyclohexasiloxane, dodecamethyl/10911	6.43 /1.14	$C_{22}H_{48}OSi$	No activity reported
Sucrose/5988	8.51 /41.07	$C_{12}H_{22}O_{11}$	Sweetener agent (Tappy et al., 2010)
Silane /23953	10.65/ 6.75	$C_{23}H_{42}F_3NO_4Si_3$	No activity reported
Decanoic acid/13225435	14.19 /1.66	$C_{11}H_{22}O_2$	Antimicrobial activity reported
Octasiloxane /4080431	14.43 /0.47	$C_{16}H_{50}O_7Si_8$	No activity reported
Benzoic acid, 2-(dimethylamino)ethyl ester /75158	14.59 /18.24	$C_{10}H_{15}N$	Antibacterial activity (Park et al., 2001), antimicrobial additive in foods(Park et al., 2001)
Linoleic acid ethyl ester /5282184	16.56 /1.08	$C_{20}H_{36}O_2$	Antibacterial and anti-inflammatory (Ko et al., 2018)
9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione /537288	17.22 /7.11	$C_{18}H_{34}O_2$	No activity Reported
1,3-Dioxolane /12586	26.04 /3.61	$C_{22}H_{38}O_4$	Antibacterial and antifungal activity (Hatice et al, see belwo).
1,1,1,3,5,5,5-Heptamethyltrisiloxane/6327366	26.55 /1.57	$C_{16}H_{50}O_7Si_8$	Antimicrobial activity reported (Wojciechowska et al., 2017)
Hexasiloxane/87782	28.95 /1.49	$C_{12}H_{38}O_5Si_6$	No activity Reported

3.6 Molecular docking of *B. macrophylla* compounds with *C. albicans* target protein

Molecular docking was performed to study the interactions of identified phytocompounds in methanolic extract of *B. macrophylla* of with Cytochrome P450 14 alpha-sterol demethylase (1EA1). Molecular docking was performed by autoDock vina software.

Docking results showed that among all the selected compounds of *B. macrophylla* sucrose showed the highest binding energy (-6.3 KJ/mol) followed by 9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione (-5.1 KJ/mol), Cyclohexasiloxane, dodecamethyl (-4.9 KJ/mol), N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane (-4.8 KJ/mol), 1-(3-Hydroxypropyl)-2-piperidinone (-4.5 KJ/mol), Benzoic acid, 2-(dimethylamino) ethyl ester (-4.4 KJ/mol), 5-Keto-D-fructose (-4.3 KJ/mol) and 1,3-Dioxolane (-2.6 KJ/mol). Sucrose makes hydrogen bonds with GLU, ARG, HIS, TYR at positions 271, 274, 275, 426 respectively, Sucrose also showed hydrophobic interactions with TRP, LEU, HIS, HIS, PHE, GLU, ARG at position 267, 317, 318, 363, 365, 424, 427 respectively. Interactive amino acids are shown in Table 5 and Figure 5. 3D structures of protein ligand complexes are shown in Figure 6. Furthermore, all the selected phytocompounds were screened for drug likeness and ADME/T. Similar to our study, Kumar et al. (2020); Nidhi et al. (2020); Rolta *et al*. (2020) and Salaria et al. (2021) studied the molecular docking of *Rheum emodi* phytocompounds with fungal target 1EA1 (Cytochrome P450 14 alpha-sterol demethylase CYP51).

Table 5
E-total, Hydrogen bonding and interacting amino acids of Phytocompounds of *B. macrophylla* in complex with 1EA1

Compounds/Drugs	1EA1		
	Affinity (kcal/mol)	H-bonding	Interactive amino acids
1-(3-Hydroxypropyl)-2-piperidinone	-4.5	ARG 354	ILE 27, TRP 267, LEU 317, HIS 318, PRO 319, ALA 350, ARG 427, ASN 428, HIS 430
1,3-Dioxolane	-2.6	ARG 274	GLU 271, LEU 317, HIS 363, ASP 364, PHE 365
5-Keto-D-fructose	-4.3	ARG 23, ARG 427	THR 24, ASP 25, PRO 26, ILE 27, ASN 428, ASP 429, SER 431,
9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione	-5.1	ARG 274, ARG 354	TRP 267, HIS 318, TYR 426, ARG 427, ASN 428, HIS 430
Benzoic acid, 2-(dimethylamino) ethyl ester	-4.4	ARG 274, AGR 354	TRP 267, HIS 275, GLU 271, LEU 317, HIS 318, HIS 363, ASP 364, PHE 365, TYR 426, ARG 427, ASN 428
Cyclohexasiloxane, dodecamethyl	-4.9	ARG 354	ILE 27, TRP 267, LEU 317, HIS 318, HIS 363, GLU 424, ARG 427, ASN 428, HIS 430
N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane	-4.8	AGR 354	ILE 27, ARG 274, LEU 317, HIS 318, PRO 319, ILE 322, ALA 350, ASN 428, HIS 430
Sucrose	-6.3	GLU 271, ARG 274, HIS 275, TYR 426	TRP 267, LEU 317, HIS 318, HIS 363, PHE 365, GLU 424, ARG 427
Fluconazole	-7.2	ARG 96	LEU 100, PHE 255, HIS 255, HIS 259, VAL 434, MET 433, PHE 78, LEU 321, TYR 76, GLN 72, HIS 392, PHE 83, ALA 256, MET 79

3.7 Drug-likeness prediction of *B. macrophylla* compounds

Drug-likeness of *B. macrophylla* compounds was predicted by using Molinspiration (server) which is based on Lipinski rule of 5. Except Octasiloxane and hexasiloxane all other compounds follow the Lipinski's rule of. Whereas Sucrose, Linoleic acid ethyl ester and 1,1,1,3,5,5,5-Heptamethyltrisiloxane showed one violation which is acceptable (Table 6).

Table 6
Drug likeness prediction of *B. macrophylla* phytocompounds

Phytocompounds	miLogP	TPSA	natoms	MW	nON	nOHNH	nviolations
5-Keto-D-fructose	-3.90	115.05	12	178.14	6	4	0
N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane	1.43	48.01	14	203.04	5	0	0
1-(3-Hydroxypropyl)-2-piperidinone	0.21	40.54	11	157.21	3	1	0
α-D-Glucopyranoside	0	55.40	17	250.29	6	0	0
Cyclohexasiloxane, dodecamethyl	2.24	55.40	24	444.93	6	0	0
Sucrose	-3.75	189.53	23	342.30	10	8	1
Silane	1.12	0	1	32.12	0	0	0
Decanoic acid	8.76	26.30	29	410.67	2	0	1
Octasiloxane	9.02	142.03	61	987.71	13	2	3
Benzoic acid, 2-(dimethylamino)ethyl ester	2.14	29.54	14	193.25	3	0	0
Linoleic acid ethyl ester	7.54	26.30	2	308.51	2	0	1
9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione	0.45	52.61	15	212.25	4	0	0
1,3-Dioxolane	0.13	18.47	5	74.08	2	0	0
1,1,1,3,5,5,5-Heptamethyltrisiloxane	5.51	18.47	12	221.50	2	0	1
Hexasiloxane	4.80	175.45	53	879.37	19	0	2

3.8 Toxicity prediction of *B. macrophylla* compounds

Toxicity of *B. macrophylla* compounds was predicted with the help of PROTOX-II server, toxicity results are summarized in Table 7. Hepato-toxicity was found in active in all the selected compound, Carcino-genecity was active only N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane, Hexasiloxane, Silane, Linoleic acid ethyl ester and 1,3-Dioxolane, immunetoxicity was found inactive in all the selected compounds, Mutagenicity was active only in N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane, Cytotoxicity was inactive in all the selected compounds of *B. macrophylla* (Table 7). Similar to our study, Rolta et al. 2020c analyse the drug likeness and toxicity of *Rheum emodin* phytocompounds by using Molinspiration and PROTOX-II server, Similarly, Salaria et al. 2020 studied the toxicity of *Thymus serpyllum* phytocompounds by using PROTOX-II server. In literature several of *insilico* drug likeness and toxicity was predicted by using Molinspiration and PROTOX-II server Supandi et al. 2018; Ghosh et al. 2019 and Tolosa et al. 2020.

Table 7
Toxicity prediction of *B. macrophylla* phytocompounds

Phytocompounds	Protox-II					
	LD50 (mg/kg)	Hepatotoxicity	Carcino-genecity	Immuno toxicity	Muta-genicity	Cyto-toxicity
5-Keto-D-fructose	23000 (class 6)	Inactive	Inactive	Inactive	Inactive	Inactive
N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane	2492 (Class 5)	Inactive	Active	Inactive	Active	Inactive
1-(3-Hydroxypropyl)-2-piperidinone	41 (Class 2)	Inactive	Inactive	Inactive	Inactive	Inactive
α-D-Glucopyranoside	648 (Class 3)	Inactive	Inactive	Inactive	Inactive	Inactive
Cyclohexasiloxane, dodecamethyl	1540 (Class 4)	Inactive	Inactive	Inactive	Inactive	Inactive
Sucrose	29700 (Class 6)	Inactive	Inactive	Inactive	Inactive	Inactive
Silane	3160 (Class 5)	Inactive	Active	Inactive	Inactive	Inactive
Decanoic acid	10000 (Class 6)	Inactive	Inactive	Inactive	Inactive	Inactive
Octasiloxane	1540 (Class 4)	Inactive	Inactive	Inactive	Inactive	Inactive
Benzoic acid, 2-(dimethylamino)ethyl ester	460 (Class 4)	Inactive	Inactive	Inactive	Inactive	Inactive

Phytocompounds	Protox-II					
	LD50 (mg/kg)	Hepatotoxicity	Carcino-genecity	Immuno toxicity	Muta-genicity	Cyto-toxicity
Linoleic acid ethyl ester	20000 (Class 6)	Inactive	Active	Inactive	Inactive	Inactive
9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione	5000 (Class 5)	Inactive	Inactive	Inactive	Inactive	Inactive
1,3-Dioxolane	3000 (Class 5)	Inactive	Active	Inactive	Inactive	Inactive
1,1,1,3,5,5,5-Heptamethyltrisiloxane	24134 (Class 6)	Inactive	Inactive	Inactive	Inactive	Inactive
Hexasiloxane	9280 (Class 6)	Inactive	Active	Inactive	Inactive	Inactive

4. Conclusion

This is the first report on systematic study of methanolic extract of *B. macrophylla* and showed antifungal activity. Methanolic extract also increased potency of fluconazole and amphotericin B by 8-128 folds. Hence combinatorial formulations of methanolic extract of *B. macrophylla* and low dosage of fluconazole and amphotericin B could be potentially used for the treatment of fluconazole and amphotericin B resistant candidiasis. GC-MS studies showed interesting findings that, major phytocompounds detected were rare sugars and their derivatives, SCFA and others phytocompounds have been reported in the literature for antimicrobial properties and food preservation properties.

Declarations

Author's contribution: All the experiments were performed by Ms. Shiwani Shukla, Ms. Anjali Kashyap and Er. Rajan Rolta. Dr. Vikas Kumar provided technical inputs. Dr Anuradha Sourirajan and Dr Kamal Dev conceived the idea and provided guidance to execute the research project.

Conflict of interest: The authors declare that they have no conflicts of interest.

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Figures

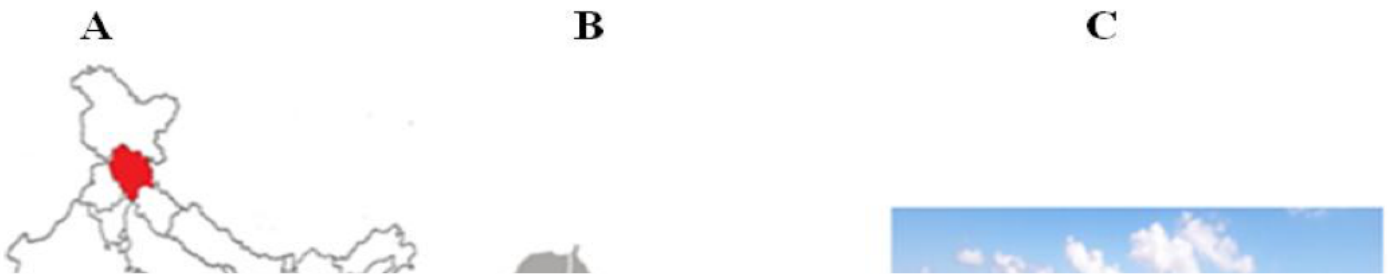

Figure 1

Geographical location of *B. macrophylla.* Map of India showing Himachal Pradesh as red shade as North Indian state (A), Map of Himachal Pradesh Showing Shimla district in blue shade (B), Natural View of Chanshal Valley, from where *B. macrophylla* was collected (C), *B. macrophylla* in natural habitat (D), and Rhizome of *B. macrophylla*used in the current study (E).

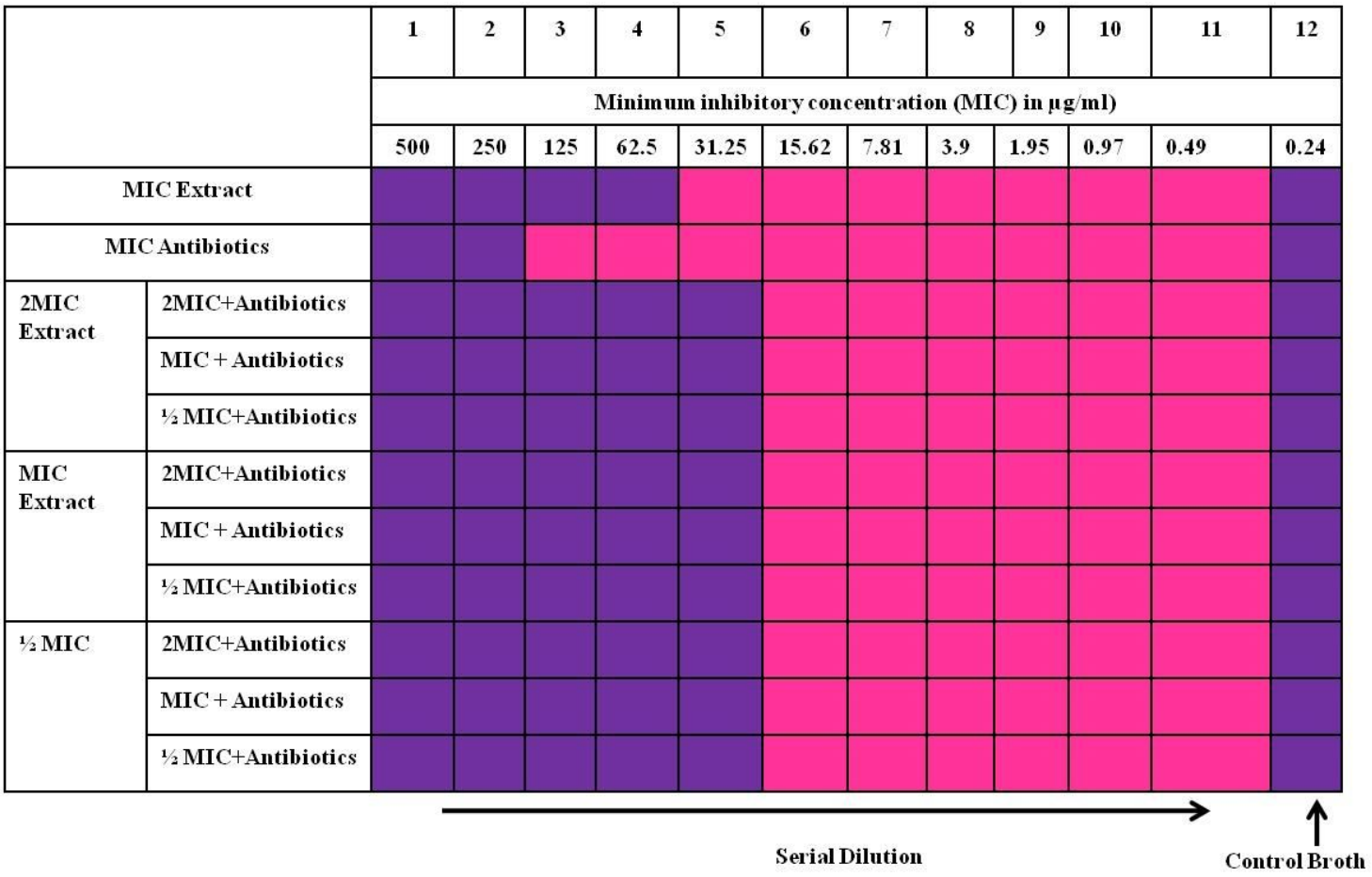

Figure 2

Schematic diagram to measure the synergistic activity between methanolic extract of rhizome of *B. macrophylla* and antibiotics (fluconazole or amphotericin B) using different dilutions. Assay was done using 96 wells micro titer plates. Purple color indicates dead cells, whereas pink color represents viable cells.

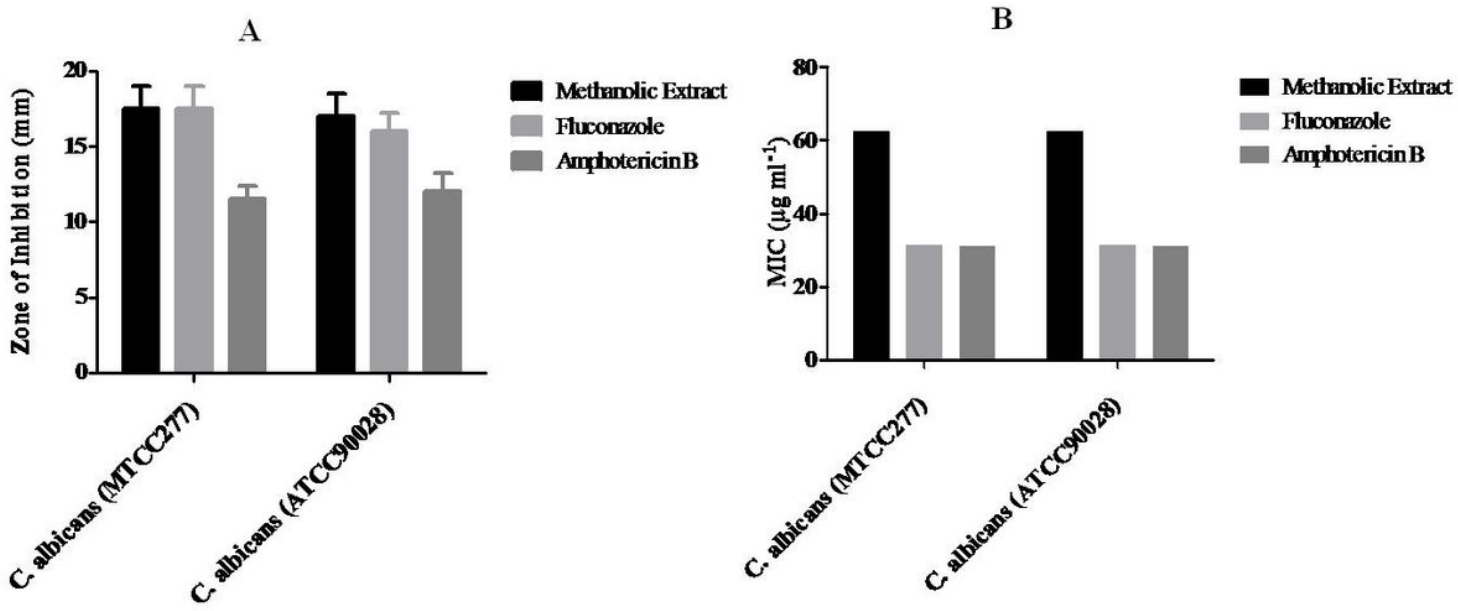

Figure 3

Qualitative and Quantitative methods to measure antifungal activity of methanolic extract of *B. macrophylla*. Methanolic extracts were subjected to: (A) antifungal activity by agar well diffusion method and (B) antifungal activity measured by broth dilution methods against *S. cerevisiae* (H1086), *C. albicans* (MTCC277 and ATCC90028). Fluconazole and amphotericin B were used as positive controls in both the assays.

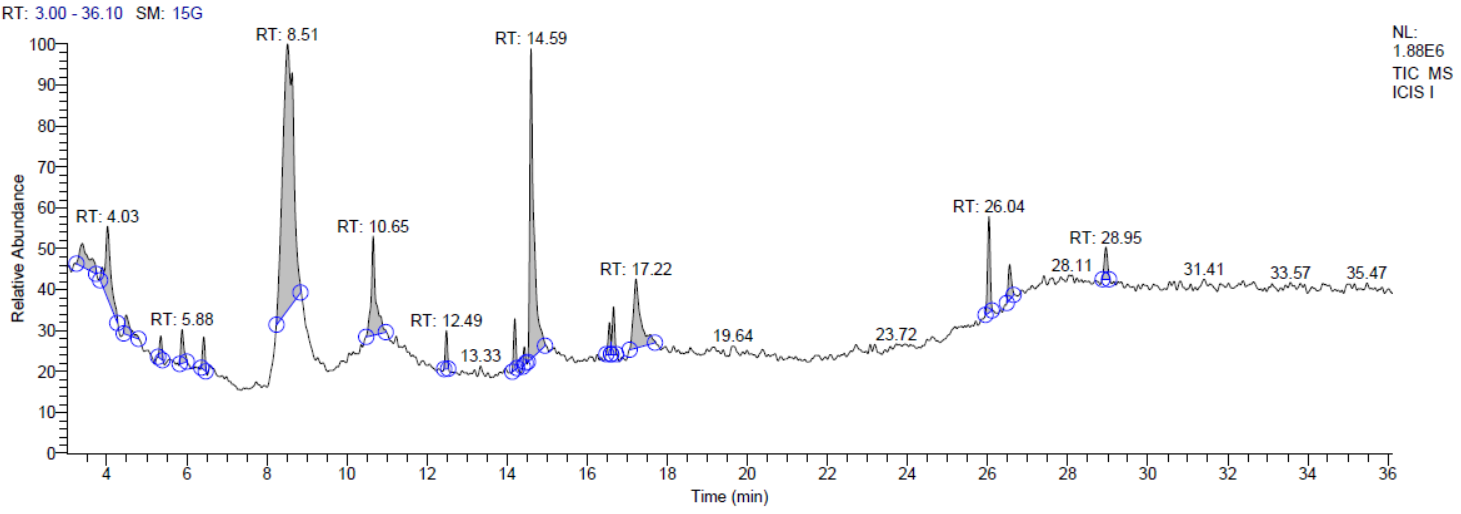

Figure 4

GC-MS fingerprint of methanolic extract of *B. macrophylla* showing Time (min) at X-axis (RT) and relative abundance at Y-axis.

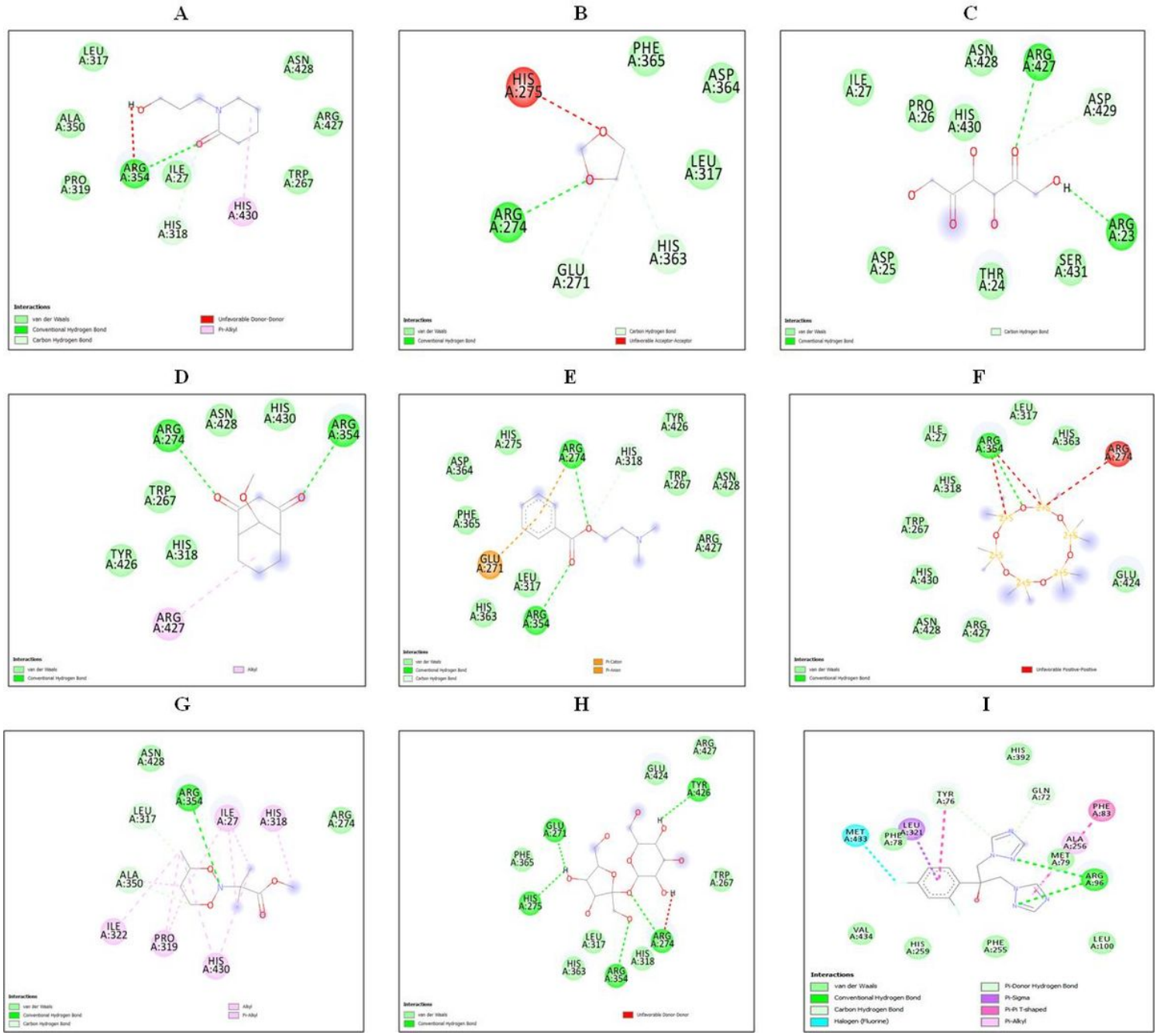

Figure 5

2D interactions of *B. macrophylla* with fungal target protein (1EA1): (A) 1-(3-Hydroxypropyl)-2-piperidinone, (B) 1,3-Dioxolane, (C) 5-Keto-D-fructose, (D) 9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione, (E) Benzoic acid, 2-(dimethylamino) ethyl ester,(F) Benzoic acid, 2-(dimethylamino) ethyl ester, (G) N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane, (H) Sucrose and (I) Fluconazole

Figure 6

3D interactions of *B. macrophylla* with fungal target protein (1EA1): (A) 1-(3-Hydroxypropyl)-2-piperidinone, (B) 1,3-Dioxolane, (C) 5-Keto-D-fructose,(D) 9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione, (E) Benzoic acid, 2-(dimethylamino) ethyl ester,(F) Benzoic acid, 2-(dimethylamino) ethyl ester, (G) N-(1-Methoxycarbonyl-1-methylethyl)-4-methyl-2-aza-1,3-dioxane, (H) Sucrose and (I) Fluconazole.

Supplementary Files

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