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Phytochemical Screening And Antifungal Activity Of Rosa Indica

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ABSTRACT:

Rosa indica's methanolic extract was created in a Soxhlet reaction, and the dry powder that resulted was dissolved in 1 mg/ml dimethyl sulfoxide (DMSO). The antifungal efficacy of these methanolic extracts was examined. The test was run on various fungus strains. Five fungal strains were examined for antifungal activity, and nearly all fungal infections showed good zones of inhibition. The methanolic extract of Rosa indica petals was used at various dosages to produce zones of inhibition with diameters ranging from 10 mm to 14 mm. The various phytochemical analyses of the methanolic extract of Rosa indica flower petals produced positive findings, proving the existence of these phytoconstituents. Alkaloids, anthraquinones, flavonoids, glycosides, saponins, steroids, tannins, terpenoids, and other secondary metabolites are found in plants. The goal of the current study is to determine the antifungal efficacy of the methanolic extracts of Rosa indica and to determine the existence of secondary metabolites.

Keywords: Antifungal, Rosa indica, phytoconstituents, methanolic extracts.

INTRODUCTION:

Rosa indica is a member of the Rosaceae family. The plant blooms every year, is widely available in India and all over the world in large quantities, and is found in practically all gardens to improve their beauty. The plant is a perennial, upright shrub with thorns. It contains essential oils (1%), acyclic monoterpene alcohols, geraniol (up to 75%), citronellol (20%), nerol (20%), and long-chain hydrocarbons like nonadecane or heneicosane (up to 10%), among other distinctive elements[1]. It doesn't exhibit the same colour gradations as either Rosa chinensis spontanea or 'Slater's Crimson,' though. Additionally, Redouté created a rose known as Rosa indica, La Bengale bichonne. 'Slater's Crimson China, also known as Rosa chinensis semperflorens, and Le Rosier du Bengale, according to Graham Thomas, may be this double rose [2].

'Old Blush' is a name that is commonly used to describe Redouté's Rosa indica vulgaris,

usually referred to as Common China. In any case, because they travelled to Europe via Bengal, some Chinese were given the nickname "Bengal Roses." According to Roy Shepherd, Slater even marketed his Crimson China as "Bengal Rose." Their roots, which have been lost or kept secret for a long time, can be overshadowed by the actual gift of their coming [3].

MATERIALS AND METHODS:

Collection of plant material

The plants were procured from Unani practitioners. Plant parts were separated, cleaned, washed with water and air-dried. The dried plants stored in air-tight containers until used.

Preparation of plant extract

Different plants were prepared as organic solvent extracts. With a few minor alterations, the extracts were made in accordance with Alade and Irobi (1993). 30 g of powdered plant material was soaked in 100 ml of distilled water for 72 hours at room temperature and in darkness to create the aqueous extract [4]. At set intervals, the mixture was frequently stirred. The mixture was then concentrated in vacuo after being filtered using Whatman Filter Paper No. 1. Methanol was used as the organic solvent in this study. The produced extracts were kept in aliquots and kept at 4 °C until needed.

Fungal Strains

The fungal strains used for the experiments were also purchased .The fungal strains used in the present study are as follows:

- Aspergillus flavus
- Aspergillus parasiticus
- Aspergillus niger
- Candida albicans
- Saccharomyces cervisiae

Culture media Composition of different media

The culture media used for anti-microbial susceptibility testing of the bacterial andfungal strains in the present study are as follows:

- Nutrient Broth
- Nutrient Agar
- Potato Dextrose Broth
- Potato Dextrose Agar

A common microbiological growth medium for the ordinary cultivation of undemanding

bacteria is nutrient agar. Because it maintains its solidity even at quite high temperatures, it is beneficial. Additionally, the surface of the nutrient agar supports the growth of tiny colonies of bacteria. The bacteria in nutritional broth grow in the liquid and are perceived as a soupy material rather than as distinct clumps. Typically, nutrient agar contains 0.5 g of peptone, 0.3 g of beef extract, 1.5 g of agar, and 0.5 g of sodium chloride. The final volume is made up of 100 ml of distilled water. At 37 °C, the pH is set to neutral (6.8). With the exception of leaving out the agar, the composition and preparation of nutritional broth are both the same [5].

Common microbiological media produced from potato infusion and dextrose (corn sugar) include potato dextrose agar (PDA) and potato dextrose broth (PDB) [6]. The most popular medium for cultivating fungus and bacteria that attack living plants or decompose dead plant tissue is potato dextrose agar. Boiling 300g of sliced (washed but unpeeled) potatoes in water for 30 minutes can be used to make potato infusion. The broth should then be decanted or strained through cheesecloth. To make the suspension a total of one liter, distilled water is added. After adding 20g of dextrose and 20g of agar agar powder, the medium is autoclaved at 15 psi for 15 minutes to sterilize it. Yeasts like Candida albicans and Saccharomyces cerevisiae, as well as molds like Aspergillus niger, can both be cultivated on PDA [7].

Preparation of Inoculum Growth Method

At least three to five well-isolated colonies with the same morphological type are chosen from an agar plate culture before the growth procedure is carried out. The growth is transferred into a tube holding 4 to 5 ml of nutrient broth by touching the top of each colony with a loop. The broth culture is incubated at 37°C for the entire night until it meets or exceeds the 0.5 McFarland criterion for turbidity. To get a turbidity visually equivalent to the 0.5 McFarland standard, the turbidity of the actively growing broth culture is adjusted using sterile saline or broth. As a result, an approximate suspension is produced [8]. The inoculum tube and the 0.5 McFarland standard can be visually compared to a card with a white background and striking black lines. However, proper lighting is required.

Preparation of dried filter paper discs

The preparation of 6 mm-diameter discs, which are then put in a Petri dish and sterilized in a hot air oven, uses Whatman filter paper no. 1. Later, the experiments used the sterilized discs.

Antibiotic discs

Antibiotic discs are paper discs with antibiotic impurities that are used in antibiotic testing. The antifungal discs were obtained from Hi-Media along with the antibiotic discs. Both Nystatin (100 mcg) and Clotrimazole (10 mcg) were utilized as antifungal discs.

Evaluation of anti-fungal activity of plant extracts

Both the classic Disc diffusion method of Baur et al. (1966) and the agar well diffusion method (Perez et al., 1990) were used for the anti-fungal experiment. On Potato dextrose agar (PDA) plates, 0.5 ml (105 CFU/ml) of diluted inoculums of the test organism were applied. For well diffusion, 8 mm-diameter wells were punched into the agar medium and filled with various plant extract concentrations [9]. At 38°C, the plates were incubated for 48 hours. By assessing the zone of inhibition against the test organism, antifungal activity was assessed. The diameter of the zone of inhibition is measured, compared to the recommended antibiotics, and the results are then evaluated.

Phyto-chemical Analysis of Plant

The presence of the phytochemicals in the plant extracts was evaluated qualitatively. The phytochemicals were quantitatively estimated [10].

Qualitative Analysis

Test for the presence of Alkaloids (Wagner's test)

2 grams of iodine and 6 grams of KI are dissolved in 100 milliliters of water to create Wagner's reagent. 500 mg of plant material were extracted in a water bath for 20 minutes using 500 cc of methanol. After filtering off the extract, it was allowed to cool. 2 ml of the extract were taken, and a few drops of Wagner's reagent were added. Alkaloids are present when a reddish-brown precipitate forms.

Test for the presence of Anthraquinone (Borntrager's test)

In a water bath, 0.5 g of the extracts were heated for a short time in 10% HCl. After filtering, it was allowed to cool. CHCl3 in an equal volume was added to the filtrate. The mixture was heated while a few drops of 10% NH were added. Anthraquinones are indicated by the formation of rose-pink hue.

Test for the presence of Flavonoids

Over a steam bath for three minutes, a piece of coarse powder was cooked with 10 cc of ethyl acetate. After filtering the mixture, a yellow colour was seen when 4 milliliters of the filtrate were mixed with 1 milliliter of diluted ammonia solution.

Test for the presence of Phlobatannins

To see the formation of a red precipitate, an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid (HCl).

Test for the presence of Glycosides (Fehling's test)

In 5 ml of methanol, 0.5 g of unrefined powder was dissolved. In a test tube, 10 ml of 50% HCl was added to 2 ml of methanolic extract. For 30 minutes, the mixture was boiled in a bath of boiling water. In order to detect the presence of glycosides, 5 ml of Fehling's solution was added, and the mixture was heated for 5 minutes. A brick red precipitate was then seen.

Test for the presence of Saponins (Frothing test)

The extract was heated to boiling after being agitated with 5 ml of distilled water weighing around 0.2 g. The presence of saponins is indicated by foaming, which has a creamy consistency and tiny bubbles.

Test for the presence of Steroids (Salkowski test)

One milliliter of the extract was mixed with five drops of concentrated H2SO4, and red colour indicated the presence of steroids.

Tannins (Ferric chloride test)

10 cc of distilled water were added to 0.5 g of the raw powder and mixed. This was filtered, and after adding ferric chloride reagent to the filtrate, a blue-black precipitate was observed as proof that tannin was present.

Test for the presence of Terpenoids (Salkowski test)

Concentrated H_2 $S0^4$ (3 ml) was carefully added to produce a layer after 0.2 g of the plant extract was carefully combined with 2 ml of chloroform. The interface developed a reddish brown coloring to signify the presence of terpenoids, which produced positive outcomes.

Quantitative Analysis

Estimation of total phenols by gallic acid assay

Folin Ciocalteu reagent was used to calculate total phenols (McDonald et al., 2001). Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1 M) were combined with a dilute extract of each plant (0.5 ml of 1:10 g ml-1) or gallic acid (standard phenolic component). The total phenols were calculated using colorimetry at 765 nm after the mixes were let to stand for 15 min. Gallic acid solutions in methanol and water (50:50, v/v) solutions of 0, 50, 100, 150, 200, and 250 mg L-1 were used to create the standard curve. Gallic acid equivalent (mg g -1 of dry mass), which is a typical reference compound, is used to express total phenol values[11,12].

Estimation of total tannins by tannic acid assay

The Folin-Denis reagent method is used to estimate the total tannins. For a thorough extraction of tannins, 2g of air-dried powder samples were refluxed with 75 ml of double-distilled water for 30 minutes. After cooling, the entire mixture was filtered using Watmann filter paper number 1. Centrifuging the filtrate took 20 minutes at 2000 rpm. Supernatant was collected in a 100 ml volumetric flask, and the volume was increased to 100 ml using distilled water in two separate glasses. Tannic acid solution was the "blank" utilized in the protocol [13].

RESULTS AND DISCUSSION:

Antifungal activity of Rosa indica

The methanolic extract of Rosa indica flower petals was made in a Soxhlet apparatus, and the dry powder that resulted was dissolved in 1 mg/ml dimethyl sulfoxide (DMSO). When

the plant extract underwent the antifungal experiment, significant zones of inhibition were seen. Following a 72-hour incubation period at room temperature, the zones were measured for diameter, and comparisons were made with anti-fungal discs obtained from Hi.-Media. The test was run on various fungus strains, including Candida albicans, Aspergillus flavus, Aspergillus niger, Aspergillus parasitic, and Saccharomyces cervisiae. The plant extract was toxic to the fungi at concentrations of 100 g, 200 g, 300 g, and 500 g.

All fungal strains were examined for antifungal activity, and nearly all fungal infections showed good zones of inhibition. The methanolic extract of Rosa indica petals was used at various dosages to produce zones of inhibition with diameters ranging from 10 mm to 14 mm.

At all four doses of the methanolic extract of Rosa indica petal, the zone of inhibition for Aspergillus flavus was discovered. The zones of inhibition were highly distinct, measuring between 10 and 14 mm, and they got bigger as the methanolic extract of Rosa indica petals got stronger. After growth was inhibited by 100 g of a methanolic extract of Rosa indica petals, the zone of inhibition's diameter was 10 mm. It was determined that the zone of inhibition identified at 200 g had a diameter of 12 mm, which was greater than the zone diameter seen at 100 g. Both the 300 g and 500 g doses of the methanolic extract of Rosa indica petals had 14 mm-diameter zones of inhibition.In the case of Aspergillus niger, when it was discovered to be ranging from 10 mm to 14 mm, the range of the width of the zone of inhibition was greater. At each of the four doses of the methanolic extract of Rosa indica petals, the zones of inhibition were visible. The zone of inhibition was measured to be 10 mm wide at 100 g, but it grew to 12 mm in diameter at 200 g. At doses of 300 g and 500 g of plant extract, the diameter of the zone of inhibition was determined to be 14 mm.

Table 1:Anti-fungal activity of Rosa indica methanolic plant extract at different concentration

S.No.	Fungal strains	Zone of inhibition (mm)			
1	Aspergillus flavus	100 (mg)	200 (mg)	300 (mg)	500 (mg)
2	Aspergillus niger	10	12	14	14
3	Aspergillus parasiticus	10	12	14	14
4	Candida albicans	-	-	12	12
5	Saccharomyces cervisiae	10	12	12	14

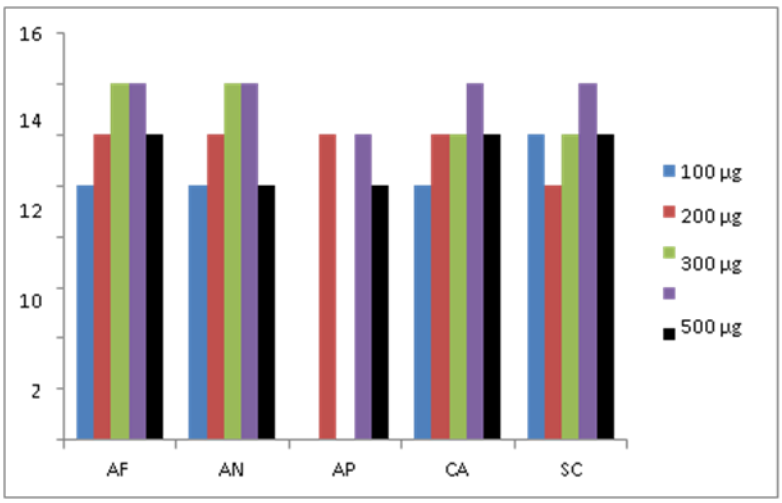

Fig. 1: The comparative results of all the anti-fungal activity of the methanolic seed extract of Rosa indica against the fungal strains

Only two of the four doses of the methanolic extract of Rosa indica petals showed a zone of inhibition for Aspergillus parasiticus. At 200 g and 500 g of methanolic extract of Rosa indica petals, the inhibitory zones were visible. At concentrations of methanolic extract of 100 g and 300 g, there was no zone of inhibition.

Both times, when the concentration of the methanolic extract of Rosa indica petals was 200 g and 500 g, the zone of inhibition diameter was measured to be 12 mm.

For Candida albicans, there were highly distinct zones of inhibition discovered. All four methanolic extract concentrations of petals from Rosa indica, with diameters ranging from 10 to 14 mm, prevented fungal growth. The zone of inhibition for the methanolic extract of Rosa indica petals was 10 mm at 100 g and 12 mm at both 200 g and 300 g concentrations. 500 g of Rosa indica flower petals' methanolic extract revealed a 14-mm zone of inhibition.

All four amounts suppressed Saccharomyces cervisiae as well. At 100 and 300 g, the zones of inhibition were discovered to be 12 mm. The methanolic extract of Rosa indica petals' zone of inhibition was found to have a diameter of 10 mm at a concentration of 200 g and 14 mm at a concentration of 500 g (Table 1).

Phyto-chemcial analysis of Rosa indica

To determine the existence of secondary metabolites, the methanolic extract of Rosa indica flower petals underwent a qualitative analysis. Alkaloids, anthraquinones, flavonoids, phlobatanins, glycosides, saponins, steroids, tannins, terpenoids, and other secondary metabolites are found in plants. The various phytochemical analyses of the

methanolic extract of Rosa indica flower petals produced positive findings, proving the existence of these phytoconstituents.

Wagner's method was used to analyse the alkaloids in 2 ml of methanolic extract of Rosa indica petals after adding a few drops of reagent Wagner. The methanolic extract of Rosa indica flower petals showed signs of alkaloids in the form of a reddish-brown precipitate.

The examination of anthraquinones in the methanolic extract of Rosa indica flower petals was done using Borntrager's assay. A few minutes were spent boiling 5 ml of methanolic flower extract in a water bath with 2 ml of 10% HCl. After filtering, the mixture was allowed to cool. The filtrate was then combined with an equivalent volume of CHCl3. The presence of anthraquinones in the methanolic extract of Rosa indica flower petals was confirmed by the creation of a rose-pink colour after adding ammonium solution to the mixture and heating it.

The presence of flavonoids was also confirmed in the methanolic extract of Rosa indica flower petals by boiling it for three minutes over a steam bath while adding 10 ml of ethyl acetate. After filtering the mixture, the filtrate was mixed with 1 ml of diluted ammonia solution, which resulted in a yellow colour as a sign that the flavonoids were present and had passed the test.

The presence of plobatanins in the methanolic extract of Rosa indica flower petals was investigated. When the methanolic extract of Rosa indica petals was boiled with 1% aqueous HCl, a red precipitate was seen, indicating its presence.

To determine whether glycosides were present in the methanolic extract of Rosa indica flower petals, Fehling's test was used. 10 ml of 50% HCl and 5 ml of a methanolic extract of Rosa indica flower petals were combined in a test tube. For 30 minutes, the mixture was boiled in a bath of boiling water. After including 5 ml of Fehling's solution, the mixture was cooked for 5 minutes. The presence of glycosides in the methanolic petal extract of Rosa indica was confirmed by the development of a brick-red precipitate.

By using a ferric chloride test, tannins were determined to be present. The presence of a blue-black precipitate was interpreted as proof that tannins were present in the Plantago ovata methanolic seed extract when it was combined with the ferric chloride reagent.

To validate the presence of saponins, Frothing's test was run. Plantago ovata methanolic seed extract equivalent to 2 ml was agitated with 5 ml of distilled water before being heated to boiling. The presence of saponins in the methanolic extract of Rosa indica flower petals is indicated by foaming (the appearance of a creamy foam with tiny bubbles).

Terpenoids were checked using the Salkowski test. To create a layer, the methanolic extract of Rosa indica flower petals was combined with 2 ml of chloroform and 3 ml of strong sulfuric acid. The methanolic extract of Rosa indica flower petals developed a reddish brown hue at the interface, indicating the presence of terpenoids.

The methanolic extract of Rosa indica flower petals underwent the Salkwoski test to check for the presence of steroids. The emergence of a red colour after adding 5 drops of concentrated H2SO4 to 1 ml of extract reveals the presence of steroids in the methanolic extract of Rosa indica flower petals.

Rosa indica petals were found to have all of the phytoconstituents. This explains why plant extracts have antifungal properties (Table 2).

Table 2. Phyto-chemical analysis of Rosa indica

Active principle	**Test**	**Result**
Alkaloids	Wagner's	+
Anthraquinones	Borntrager's	+
Flavonoids	NaOH	+
Phlobatanins	HCl	+
Glycosides	Fehling's	+
Saponins	Frothing	+
Steroids	Salkwoski	-
Tannins	Ferric chloride	+
Terpenoids	Salkwoski	+

Determination of total phenols and tannins

Using the Folin-Ciocalteau Reagent, the total phenolic content was calculated spectrophotometrically at 765 nm. The concentration of gallic acid in the methanolic extract of Rosa indica flower petals was determined, and a calibration curve was developed using gallic acid as the reference. The dilution factor was multiplied by the observed concentrations. Gallic Acid Equivalent, or GAE, was used to express the results (in mg/g of dry mass).

Since this assay analyses all phenolics, Gallic acid was chosen as the standard because it is both a stable and pure material that is also less expensive than alternative options. We have also studied the stability of conventional solutions of gallic acid, and we can say that when stored tightly covered and refrigerated, they lose less than 5% of their value over the course of two weeks. The estimated GAE for the methanolic extract of Rosa indica flower petals was 10.81 GAE/g.

Tannic Acid Equivalents per Gramme, or TAE (mg/g of dry mass), were determined by spectrophotometric analysis at 760 nm using the Folin-Denis Reagent and the Tannic Acid calibration standard. The estimated TAE for the methanolic extract of Rosa indica flower petals was 2.98 TAE/g.

CONCLUSION:

The current study also assessed the crude methanolic extract of Rosa indica flower petals' anti-fungal properties. It was discovered that practically all of the fungus strains tested in the study had their growth inhibited. The doses of 300 g and 500 g, indica methanolic petal extract demonstrated the greatest suppression of the proliferation of fungal cells, with the diameter of the zone of inhibition measured at between 12 and 14 mm. With all of the fungal strains utilised in the current investigation, indica was extremely susceptible. The typical anti-fungal discs utilised in the current investigation were shown to have equivalent fungal growth inhibition, and in all strains, the diameter of the zone of inhibition was significantly larger. Consequently, the unprocessed methanolic petal extract of Rosa indica is a powerful anti-fungal agent, Rosa indica is effective against fungus infections. This is the first outcome mentioning Rosa indica's anti-fungal properties. All of the phytochemicals typically found in plants as secondary metabolites were found in the methanolic extract of Rosa indica flower petals, according to phytochemical investigations. The phytoconstituents in the methanolic extract of Rosa indica flower petals support the plant's anti-fungal properties. Through a variety of processes, including binding to adhesins and proteins in the bacterial and fungal cells, complexation with them, or intercalation into their cell walls, these phytoconstituents act on the bacterial and fungal strains to restrict their growth. The pathogenic cells' membranes are known to be disrupted and their substrate supply cut off by the phenols.

REFERENCES:

1. Alanís-Garza B.A., González-González G.M., Salazar-Arandaa R., Waksman de Torresa N. and Rivas-Galindo V.M., 2007. Screeningof antifungal activity of plants from the northeast of Mexico, Journalof Ethnopharmacology, 114:3, Pages 468-471
2. Aqil, F. and Ahmad, I. 'Broad spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants'. World I Micro. and Biotech., Vol.19, no.6, 2003 pp.653-657.
3. Braga F.G., Maria L´ucia M. Bouzada, Rodrigo L. Fabri, Magnumde O. Matos, Francis O. Moreira, Elita Scio, Elaine S. Coimbra, 2007. Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. Journal of Ethnopharmacology 111:

396–402

4. Clark, A.M., Walker, L.A. 1999. Discovery of Antifungal Agents from Natural Sources: Virulence Factor Target. pp. 95-107. In Cutler, S.J. and Cutler, H.G. Biologically Active Natural Products: Pharmaceuticals. CRC Press, Washington, DC.
5. Ahmad I, Beg AZ. 2001. Antimicrobial and Phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens J. Ethnopharm. 74: 87-91.
6. Cuenca-Estrella, M., A. Gomez-Lopez, E. Mellado, M.J. Buitrago, Monzon and J.L. Rodriguez-Tudela, 2006. Head-to-head comparison of the activities of currently available antifungal agents against 3,378 Spanish clinical isolates of yeasts and filamentousfungi. Antimicrob. Agents Chemother., 50: 917-921.
7. Dixit S.N. Tripathi and S.C. Upadhyay RR, 1976, The Antifungal Substance of rose flowers. Economic Botany Vol. 30, No. 4.
8. Gearhart, M.O., 1994. Worsening of lung infection with fluconazoleand review of azole antifungal hepatotoxicity. Annals of Pharmacotherapy 28, 117 7-1 181.
9. Gurgel LA, Sidrim JJ, Martins DT, Cechinel Filho V, Rao VS 2005. In vitro antifungal activity of dragon's blood from Croton urucurana against dermatophytes. J Ethnopharmacol; 97(2):409-412.
10. Hamza, O.J., van den Bout-van den C.J. Beukel, M.I. Matee, M.J. Moshi, F.H. Mikx, H.O. Selemani, Z.H. Mbwambo, Van der A.J. Ven and P.E. Verweij, 2006. Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections. Journal of Ethnopharmacology, 108: 124-132.
11. Krishnakumar, T, Ranjini, C.E. and Sasidharan, V.K. , 1997. Antibacterial and antifungal activity of secondary metabolites from some medicinal and other common plant species. J. Life Sci. Vol. 11. 14-19.
12. Liliana Muschietti, Marcos Derita, Valeria S¨ulsen, Juan de Dios Mu˜noz, Graciela Ferraro, Susana Zacchino, Virginia Martino, 2005. In vitro antifungal assay of traditional Argentine medicinal plants. Journal of Ethnopharmacology 102: 233–238.
13. Masoko, P., J. Picard and J.N. Eloff, 2007. The antifungal activity of twenty-four Southern African Combretum species (Combretaceae).S. Afr. J. Bot., 73: 173-183.