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ISOLATION, QUANTIFICATION AND ANTIMICROBIAL ACTIVITIES OF PHYTOSTEROLS FROM DIFFERENT PARTS OF CASSIA *PUMILA* LAMK

Ankita Yadav, Richa Bhardwaj and R. A. Sharma

Plant Physiology and Biochemistry laboratory, Department of Botany, University of Rajasthan, Jaipur, India

***Corresponding author e-mail:** yadanki22@gmail.com

ABSTRACT

Cassia species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. Aim of this study is to identify and characterize the bioactive principles from the different parts of *Cassia pumila*. Four compounds were isolated and purified and the structures were determined as β-sitosterol, lanosterol, campersterol, stigmasterol by analysis of physical, chemical and spectral characterstics (IR,NMR). Present study shows the presence of various concentrations of phytosterols in different plant parts of *C. pumila*. The higher level of total phytosterols was measured in pods of *C. pumila* (1.15 mg/g dw) and lowest in flowers (0.38mg/gdw).The highest level of β-sitosterol and lanosterol, was found highest in pods(0.23mg/gdw) and 0.24mg/gdw) respectively, whereas highest level of campersterol was found in leaves(0.24mg/gdw). The isolated phytosterols were effective against all test bacteria and fungi. β-sitosterol was more active against fungi and bacteria and their MIC value was recorded 2 X 10^3 mg/disc while the MIC value of 3X 10^3 mg/disc was recorded for lanosterol, Campersterol and Stigmasterol.

Key words: *Cassia pumila*, phytosterols, Campersterol, lanosterol, Stigmasterol, β sitosterol

INTRODUCTION

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs; prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases (Sharma *et al*, 2013). *Cassia pumila* Lamk. is a terrestrial, dwarf ,perennial herb, commonly known as Sarmal, and is found throughout the India, tropical Asia and Australia. The pulp from its fruits called cassia pulp is well known as laxative and purgative, used in treatment of constipation (Kirtikar and Basu, 1955; Bhattacharjee, 1998).

Phytosterols have been found to occur in the whole plant and all types of tissues such as roots, stem, leaves, bark, cotyledons, flowers, pollen, peel of fruits, pulp of fruits, seed, seed coat, and endosperm well including cell cultures. The work on the biochemistry of steroids /sterols has been reviewed by a number of workers (Sofowara and Hardman, 1974; Grunwald, 1980; Honda *et al.*, 2003; Uba *et al.*, 2008) and possess potentials for development of antitumor activity (Cassady and Suffness, 1980; Kumar *et al*., 2010; Gupta *et al*., 2000), hyperglycemic activity (Palanichamy *et al*., 1988), anti-inflammatory activity(Palanichamy and Nagarjan, 1990a; Narnath *et al*., 2009),. The review of literature reveals that maximum concentration of sterols was found in leaf (Hatano *et al*., 1999; Nes, 2000; Rahaman *et al*., 2006). Interestingly not much attention has been paid to investigate the genus Cassia for steroidal constitutents, except few reports of their occurrence in *C.siamea*, *C.sophera* and *C.fistula*(Varshney and Pal,1977;Ghosh *et*

al.,1982;Bhakta,2001),*C.tora* and *C.obtusifoia* (Upadhyaya and Singh,1986s), C.roxburghii(Ashok and Sharma,1987), *C.tora* and *C.hirsuta*(Miralles *et al*.,1989;Joshi *et al*.,1986).However, it is noteworthy that β-sitosterol isolated from *Cyperus rotundus*(Gupta *et al*.,1980) and *C.alanta* (Palanicharmy and Nagrajan,1990) has been described the anti-inflammatory & antipyretic properties in *C.italica*(Jain *et al*.,1997;farswan *et al*.,2009).In view of this, in the present investigation the selected cassia species have been assessed for sterols composition.

MATERIALS AND METHODS

Collection and Identification

Cassia pumila Lamk. is a weed of rainy season which is found usually in the shade of trees, crevices of rocks and also in the open gravelly substratum, often hidden amongst grasses. Plant species were collected from Jaygragh fort of Amer, and Garganesh temple at Jaipur. All the plant parts were washed with tap water, dried at room temperature and ground to fine powder. The species specimen was authenticated and submitted in herbarium, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India and got the voucher specimen No. RUBL15482.

Processing and Extraction

The all plant parts (root, stem, leaves, flowers and pods) of *Cassia pumila* and were studied for phytosterol composition. Plant samples were first defatted with petroleum ether (60°-80°C) for 24 hrs on a water bath. Later, each defatted material was dried and re-extracted, with benzene for 24 hrs. Subsequently, the benzene extract was dried in vacuo and weighed. The benzene fraction was then chromatographed on TLC (Silica gel; hexane-acetone (8:2) along with standard markers (Fazli and Hardman, 1968). The developed chromatograms were air-dried. Visualized under UV light and the fluorescence or the colours were noted. Later, each was sprayed with 50% H_2SO_4 (Bennett and Heftmimn, 1962) or anisaldehyde reagent (prepared by mixing minimum 0.5 ml anisaldehyde + ml H_2SO_4 + 50 ml glacial acetic acid; Heftmann, 1965) separately and heated to 100°C for 5-10 min until characteristic colours developed. The reaction time required for initial appearance of the colour in day light and after heating for 10 min was recorded.

Four fluorescent spots coinciding to reference lanosterol (R_f 0.97), β-sitosterol (R_f 0.90), stigmasterol(R_f 0.84) and campesterol (R_f 0.34) were isolated by TLC ,eluted and purified. Later, the isolated compounds were crystallized and identified by using MP, UV, IR and NMR spectroscopy (Veitech and Grover, 2008). Using spectrophotometric methods of Mabry *et al.* (1970) and Kariyone *et al.* (1953), the quantification was made of lanosterol, β-sitosterol, stigmasterol and campesterol.

Test Microorganisms

Standard strains of *Staphylococcus aureus, Salmonella typhyi, Escherichia coli and Pseudomonas aeruginosa* obtain from microbiology Lab. SMS Medical College, Jaipur and *Aspergillus flavus, Aspergillus. niger, Fusarium monilliformae and Rhizoctonia bataticola* were obtained from seed pathology lab, Department of Botany, University of Rajasthan, Jaipur .

Culture of test microbes

For the cultivation of bacteria, Nutrient Broth Medium (NBM) was prepared using 8% Nutrient Broth (Difco) in distilled water and agar-agar and sterilized at 15 lbs psi for 25-30 mins. Agar test plates were prepared by pouring ~15 ml of NBM into the petri dishes (10 mm) under aseptic conditions. A peptone saline solution was prepared (by mixing 3.56 g KH_2PO_4 + 7.23 g NaH_2PO_4 + 4.30 g, NaCl + 1 g peptone in 1000 ml of distilled water, followed by autoclaving) and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24 hrs. However, for the cultivation of fungi, Potato Dextrose Agar medium was prepared by mixing 100 ml potato infusion + 20 g agar + 20 g glucose, followed by autoclaving) and the test fungi were incubated at 27°C for 48 hrs and the cultures were maintained on same medium by regular subculturing. To prepare the test plates, in both bacteria and fungi, 10 to 15 ml of the respective medium was poured into the petridishes and used for screening. For assessing the bactericidal efficacy, a fresh suspension bacteria was prepared in saline solution from a freshly grown agar slant, while for fungicidal efficacy, a uniform spread of the test fungi was made using sterile swab.

Bactericidal and Fungicidal Assay

For both, bactericidal and fungicidal assays *in vitro* Disc diffusion method was adopted (Gold and Bowie, 1952) because of reproducibility and precision. The different test organism were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of whatman no. 1 paper (6 mm in diameter), which were containing 4 mg (or 0.4 ml) of the test extracts or control (0.4 g/ml) of the respective solvent or gentamycin (10 mg/ml) or mycostatin (100 units/ml) as reference separately. Such treated discs were air-dried at room temperature, to remove any residual

solvent which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 hrs, so as to allow the maximum diffusion of the compounds from the test discs into the plate and later, incubated at 37°C for 24 hrs in case of bacteria and 48 hrs at 27oC for fungi, after which the zones of inhibition could be easily observed. Three replicates of each test extract were examined and the mean values were then referred. The inhibition zone (IZ) in each case were recorded and the activity index (AI) was calculated as compared with those of their standard reference drugs (AI = inhibition zone of test sample/inhibition zone of standard).

Determination of MIC

Test for antibacterial activity

The antibacterial assay was carried out by microdilution method (Booth, 1971; Daouk *et al*, 1995; Hanel and Roether, 1998) in order to determine the antibacterial activity of compounds tested against the human pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0- 10^7 CFU/ml. The inoculum was prepared daily and stored at 4^0C until use. Dilutions of the inoculum were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum. All experiments were performed in duplicate and repeated three times.

Microdilution test

The minimum inhibitory concentrations (MICs) were determined using 96-well microtitre plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0 X10^7 cfu/ml. Compounds to be investigated were dissolved in broth LB medium (100 µl) with bacterial inoculums (1.0 X 10^5 cfu per well) to achieve the wanted concentrations (1 mg/ml). The microplates were incubated for 24 h at 48^0C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The optical density of each well was measured at a wavelength of 655 nm by Microplate reader (Perlong, ENM8602) and compared with a blank and the positive control. Gentamycin was used as a positive control (1 mg/ml DMSO). All experiments were performed in duplicate and repeated three times.

Test for antifungal activity

The micromycetes were maintained on Potato dextrose agar and the cultures stored at 4oC and sub-cultured once a month. In order to investigate the antifungal activity of the extracts, a modified micro dilution technique was used (Espinel-Ingroff, 2001). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0 – 10^7 in a final volume of 100 µl per well. The inoculums were stored at 4^0C for further use.

Dilutions of the inoculum were cultured on solid potato dextrose agar to verify the absence of contamination and to check the validity of the inoculum. Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The compounds investigated were dissolved in respective solvents (1 mg/ml) and added in potato dextrose broth medium with inoculum. The microplates were incubated for 72h at 28^0C, respectively. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

RESULTS AND DISCUSSION

In the present investigation, phytosterols profile has been studied *in vivo* of *C. pumila*, where lanosterol, β-sitosterol, stigmasterol and campesterol from different plant parts of *C. pumila* have been evaluated by chromatographic, spectroscopic and color reactions. The silica gel TLC of the fractions showed the presence of four phytosterols after spray of anisaldehyde reagent and 50% sulphuric acid (Table 1).

The compounds eluted from TLC were pooled together according to their TLC behaviour and isolate them with the solvents and evaporated yielding four phytosterols lanosterol, β-sitosterol, stigmasterol and campesterol. The spectral analyses of the active constituent, Spectra-I - lanosterol, Spectra-II - β-sitosterol and Spectra-III - stigmasterol , Spectra-IV - campesterol from the different plant parts of selected *C. pumila* are shown below: -

(a) **β-sitosterol**: brownish crystallization (mp 135-137°C)

UV light absorption MeOH: 206 sh, 268 sh, 356sh, 540sh

IR: νcm^{-1}/ max KBr: 3400 (O–H), 2700(C-H), 2210 (C≡C), 1700 (C=O), 1640 (C=C), 1610, 1570, 1510, 1450, 1400 (aromatic), 1385, 1310, 1270, 1180, 1010, 815

^{1}**HNMR** (300MHz, $CDCl_3$): 0.98 (H_1), 1.38 (H_2), 1.35 (H_3), 1.36 (H_4), 1.11 (H_5), 1.82 (H_6), 1.21 (H_7), 1.35 (H_8), 1.45 (H_9), 1.16 (H_{10}), 1.64 (H_{11}), 1.47 (H_{12}), 1.26 (H_{13}), 1.15 (H_{14}), 1.75 (H_{15}), 1.27 (H_{16}), 1.27 (H_{17}), 1.96 (H_{18}), 1.24 (H_{19}), 1.66 (H_{20}), 1.06 (H_{21}), 1.21 (H_{22}), 1.46 (H_{23}), 0.86 (H_{24}), 0.86 (H_{25}), 1.26 (H_{26}), 1.29 (H_{27}), 1.29 (H_{28}), 1.51 (H_{29}), 1.47 (H_{30})

^{13}C NMR (300MHz, $CDCl_3$): 14.5(C_1), 21.7 (C_2), 34.3 (C_3), 42.9 (C_4), 30.4(C_5), 20.2(C_6), 20.6 (C_7), 30.8 (C_8), 35.7 (C_9), 29.7 (C_{10}), 18.7 (C_{11}), 48.8 (C_{12}), 20.3 (C_{13}), 20.4 (C_{14}), 40.1 (C_{15}), 27.5(C_{16}), 295.35 (C_{17}), 36.9 (C_{18}), 36.8 (C_{19}), 32.1 (C_{20}), 26.9 (C_{21}), 36.2 (C_{22}), 364.81 (C_{23}), 269.86 (C_{24}), 25.2 (C_{25}), 257.26 (C_{26}), 34.5 (C_{27}), 19.3 (C_{28}), 23.6(C_{29})

(b) **Stigmasterol**: Reddish brown crystallization (mp 143-144°C)
UV light absorption MeOH: 228 sh, 245 sh, 322 sh, 540sh
IR: *v*cm^{-1}/ max KBr: 3480 (OH), 2950 (C-H), 1600 (C=O) 1365, 1570, 1510, 1450, 1400 (aromatic), 1385, 1310, 1270, 1180, 1010, 815
^{1}H NMR (300MHz, $CDCl_3$): 1.04 (H_1), 1.92 (H_2), 1.05(H_3), 1.54 (H_4), 1.16 (H_5), 1.15 (H_6), 1.35 (H_7), 1.54 (H_8), 1.76 (H_9), 1.48 (H_{10}), 1.23 (H_{11}), 1.14 (H_{12}), 1.45 (H_{13}), 1.25 (H_{14}), 1.29 (H_{15}), 1.16 (H_{16}), 1.21 (H_{17}), 1.28 (H_{18}), 1.66 (H_{19}), 1.06 (H_{20}), 1.29 (H_{21}), 0.86 (H_{22}), 0.86 (H_{23}), 1.29 (H_{24}), 1.29 (H_{25}), 1.29 (H_{26}), 1.51 (H_{27}), 1.47 (H_{28})
^{13}C NMR (300MHz, $CDCl_3$): 19.3 (C_1), 33.6 (C_2), 19.7 (C_3), 39.6 (C_4), 73.5 (C_5), 32.6 (C_6), 33.8 (C_7), 29.9 (C_8), 18.4 (C_9), 48.3 (C_{10}), 20.7 (C_{11}), 27.8 (C_{12}), 40.6 (C_{13}), 27.3 (C_{14}), 32.27 (C_{15}), 22.2 (C_{16}), 34.4 (C_{17}), 32.7 (C_{18}), 29.9 (C_{19}), 27.0 (C_{20}), 33.8 (C_{21}), 462.4 (C_{22}), 267.88 (C_{23}), 25.7 (C_{24}), 27.74 (C_{25}), 24.5 (C_{26}), 19.3 (C_{27}), 23.4 (C_{28})

(c) **Lanosterol**: Colourless crystallization (mp 131-133°C)
UV light absorption MeOH: 235 sh, 270 sh, 345 sh, 540sh
IR: *v*cm^{-1}/ max KBr: 3420 (OH), 2915 (C-H), 2220 (–C≡C–), 1615 (C=C), 1440 (C=O), 1365, 1570, 1510, 1450, 1400 (aromatic), 1385, 1310, 1270, 1180, 1010.
^{1}H NMR (300MHz, $CDCl_3$): 1.11 (H_1), 0.96 (H_2), 1.02 (H_3), 0.87 (H_4), 1.22 (H_5), 1.25 (H_6), 1.03 (H_7), 1.67 (H_8), 1.49 (H_9), 1.24 (H_{10}), 1.17(H_{11}), 1.48 (H_{12}), 1.28 (H_{13}), 1.25 (H_{14}), 1.16 (H_{15}), 1.24 (H_{16}), 1.24 (H_{17}), 1.66 (H_{18}), 1.06 (H_{19}), 1.16 (H_{20}), 1.06 (H_{21}), 2.1 (H_{22}), 0.86 (H_{23}), 1.29 (H_{24}), 1.24 (H_{25}), 1.25 (H_{26}), 1.16 (H_{27}), 1.43 (H_{28}), 1.46 (H_{29})
^{13}C NMR (300MHz, $CDCl_3$): 22.5 (C_1), 6.24 (C_2), 22.6 (C_3), 39.5 (C_4), 24.3 (C_5), 35.7 (C_6), 30.8 (C_7), 18.9 (C_8), 43.9 (C_9), 33.8 (C_{10}), 15.0 (C_{11}), 27.4 (C_{12}), 22.8 (C_{13}), 22.3 (C_{14}), 31.9 (C_{15}), 22.5 (C_{16}), 33.0 (C_{17}), 27.1 (C_{18}), 31.8 (C_{19}), 30.8 (C_{20}), 15.1 (C_{21}), 46.6 (C_{22}), 24.6 (C_{23}), 25.8 (C_{24}), 29.79 (C_{25}), 50.0 (C_{26}), 11.5 (C_{27}), 29.1 (C_{28}), 19.0 (C_{29})

(d) **Campesterol:**Colourless powder (mp 137-138°C)
UV light absorption MeOH: 210 sh, 267 sh, 316 sh, 540sh
IR: *v*cm^{-1}/ max KBr: 3380 (OH), 2920 (C-H), 2270 (C≡C), 1760 (C=O), 1610 (C=O), 1560, 1520, 1450, 1430 (aromatic), 1395, 1310, 1270, 1180, 1010, 815
^{1}HNMR(300MHz, $CDCl_3$): 1.05 (H_1), 1.87 (H_2), 1.15 (H_3), 0.87 (H_4), 0.84 (H_5), 1.08 (H_6), 1.68 (H_7), 1.17 (H_8), 1.44 (H_9), 1.65 (H_{10}), 1.17 (H_{11}), 1.27 (H_{12}), 1.28 (H_{13}), 1.69 (H_{14}), 1.04 (H_{15}), 1.25 (H_{16}), 0.88(H_{17}), 0.88 (H_{18}), 1.27 (H_{19}), 1.25 (H_{20}), 1.69 (H_{21}), 1.54 (H_{22}), 1.46 (H_{23}), 1.48 (H_{24}), 1.25 (H_{25}), 1.27 (H_{26}), 1.01 (H_{27})
^{13}C NMR (300MHz, $CDCl_3$): 17.8 (C_1), 38.9 (C_2), 17.5 (C_3), 42.3 (C_4), 21.3 (C_5), 25.8 (C_6), 31.9 (C_7), 30.9 (C_8), 18.8 (C_9), 48.2 (C_{10}), 20.7 (C_{11}), 44.4 (C_{12}), 40.1 (C_{13}), 27.4 (C_{14}), 25.20 (C_{15}), 22.5 (C_{16}), 34.2 (C_{17}), 32.4 (C_{18}), 29.8 (C_{19}), 27.8 (C_{20}), 38.3 (C_{21}), 42.6 (C_{22}), 27.7 (C_{23}), 25.9 (C_{24}), 29.74 (C_{25}), 34.7 (C_{26}), 19.4 (C_{27}), 23.8 (C_{28}), 30.6 (C_{29}), 9.8 (C_{30}).

While assessing the levels of phytosterols in different plant parts of *C.pumila* ,maximum levels of total phytosterols was measured in pods(0.78 mg/gdw) and minimum levels in flowers(0.38 mg/gdw)(Table 2). According to the nonadaptive hypothesis, the distribution of secondary metabolites within organs may be roughly equivalent to the distribution of the primary metabolic pathways responsible for the creation of the secondary metabolite (as a byproduct) and thus they do not necessarily have an adaptive function in each organ (Eriksson & Ehrlen, 1998).
Table 3 showed that the isolated phytosterols were effective against all tested bacteria and fungi but β sitosterol showed higher activity against *S.aureus* (IZ= 28.00 mm) and minimum activity was recorded against *A.niger* and *F.moniliforme* (IZ= 8.00). Similarly, Lanosterols were highly effective against *E.coli* (IZ= 18.00). The campersterol and stigmasterol was not effective comparative to β-sitosterol and lanosterol.
The antimicrobial activity of isolated four compounds were carried out by microdilutiom method against selected fungal and bacterial microorganisms, and compared with commercially available antibiotics. Basically, the MIC value indicates the potential of each extract to inhibit the microbial growth at lowest concentration. Table 4 shows the MIC for isolated phytosterols against test micro organisms recorded in mg/disc of the diametrical sections of the respective zones of inhibition for each metabolite. β-sitosterol was more active against fungi and bacteria and their MIC value was recorded 2 X 10^3 mg/disc while the MIC value of 3X 10^3 mg/disc was recorded for lanosterol, campersterol and Stigmasterol.
Significant development of a satisfactory chemical assay for the drug has been long laid by previous lack of knowledge of the precise active principles of the

drug (Trease and Evans, 1978). Although, *Cassia* species have been of much interest to the phytochemists for the isolation of their constituents but, such a comparative study involving the intact plant is first of its kind.

There are still many *C. pumila* phytosterols and their derivatives, whose pharmacological activities have not yet been investigated. It is possible that they may contain beneficial pharmacological properties. Therefore, *in vivo* and *in vitro* investigations regarding their effects could provide insight into the benefits of *C. pumila* for future clinical management of many human diseases.

Table 1: Chromatographic data and colour reaction of isolated phytosterols from *Cassia pumila*

Phytosterols	R_f×100 in solvents			Colour after heating 50% sulphuric acid in day light				Anisaldehy de reagent	M.P (in °C)
	S_1	S_2	S_3	Min.	Initial	Final	In UV		
β-sitosterol	86	90	71	1.25	GY-PK	PU-BN	DL-RD	PU	135-137
Stigmasterol	84	64	65	0.50	PK	GY	DK-BU	PU	131-133
Lanosterol	93	96	71	1.25	PK	PK-BN	DK-BN	PK	143-144
Campesterol	23	23	21	1.25	PK	GY	DL-BN	BU	137-138

Abbreviation: S_1= Hexane: Acetone (8:2); S_2= Benzene: Acetone (2:1); S_3= Benzene: Ethyl acetone(3:2); BN=Brown, GY= Grey; Bu= Blue; PK=Pink; DK= Dark; PU=Purple; DL=Dull; RD=Red

Table 2: Phytosterols contents (mg/gdw*) isolated different plant parts from *Cassia pumila*

Plant Species	*C. pumila*					
	Root	**Stem**	**Leaves**	**Flower**	**Pods**	**Total**
β-sitosterol	0.04	0.03	0.14	0.05	0.23	0.49
Stigmasterol	0.18	0.15	0.18	0.14	0.18	0.83
Lanosterol	0.11	0.13	0.14	0.04	0.24	0.66
Campesterol	0.12	0.13	0.24	0.15	0.13	0.77
Total	0.45	0.44	0.70	0.38	0.78	2.75

Table 3: Bactericidal and fungicidal efficacy of isolated phytosterols from *Cassia pumila*

Test organist	Phytosterols			
	**-sitosterol	**Lanosterole**	**Campesterol**	**Stigmasterol**
		A. Bacterial		
E. coli	19.00	18.00	12.00	10.00
	0.70	0.72	0.50	0.42
S. aureus	28.00	6.00	11.00	10.00
	1.25	0.18	0.45	0.40
P. aeruginosa	12.00	13.00	9.00	10.00
	0.42	0.52	0.38	0.41
S. typhi	13.00	14.00	8.00	8.00
	0.55	0.64	0.30	0.38
		B. Fungi		
A. flavus	13.00	11.00	14.00	0.16
	0.61	0.55	0.12	0.68
A.niger	8.00	13.00	11.00	11.00
	0.50	0.70	0.60	0.60
R. bataticola	17.00	8.00	9.00	5.00
	0.65	0.30	0.36	0.20
F. moniliforme	8.00	7.00	7.00	8.00
	0.38	0.24	0.24	0.30

Table 4: Zones of inhibition of different concentration (MIC) of phytosterol (mg/ml)

	β-sitosterol					Lanosterol					Campesterol					Stigmasterol				
Text organisms	**1×10^3**	**2×10^3**	**3×10^3**	**4×10^3**	**5×10^3**	**1×10^3**	**2×10^3**	**3×10^3**	**4×10^3**	**5×10^3**	**1×10^3**	**2×10^3**	**3×10^3**	**4×10^3**	**5×10^3**	**1×10^3**	**2×10^3**	**3×10^3**	**4×10^3**	**5×10^3**
Bacteria																				
E. coli	-	-	+	+	+	-	-	-	-	+	-	-	-	±	+	-	-	-	±	+
S. aureus	-	-	+	+	+	-	-	-	±	+	-	-	-	±	+	-	-	-	±	+
P. aeurginosa	-	-	+	+	+	-	-	-	±	+	-	-	-	±	+	-	-	-	±	+
S. typhi	-	-	±	+	+	-	-	-	±	+	-	-	-	±	+	-	±	-	±	+
Fungi																				
A. flavus	-	-	±	+	+	-	-	-	±	+	-	-	-	±	+	-	-	-	±	+
A. niger	-	-	±	+	+	-	-	-	±	+	-	-	-	±	+	-	-	-	±	+
F. moniliformae	-	-	±	+	+	-	-	-	±	+	-	-	-	±	+	-	-	-	±	+
R. bataticola	-	-	±	+	+	-	-	-	±	+	-	-	-	±	+	-	-	-	±	+

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