In vitro cytotoxic, antioxidant, antibacterial and antifungal activity of *Saussurea heteromalla* indigenous to Pakistan

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Abstract: Medicinal plants are proven to reveal vast promising potential providing novel drug candidates to combat health-related problems. The aim of current study is to discover new drug compounds with anti-anticancer, antioxidant, antibacterial and antifungal potential, to serve the purpose Saussurea *heteromalla* (Family: Asteraceae) indigenous to Pakistan was screened for the in vitro cytotoxicity against HeLa cells (Human cervical cancer cell line) compared to the NIH / 3T3 cells (mouse normal fibroblast cells) by performing the MTT colorimetric assay and antifungal, antibacterial and antioxidant potential by adopting standard protocols. *S. heteromalla* crude methanolic extract (CME) demonstrated strong cytotoxic potential against HeLa cells at 200µg/mL; (77.28 ±1.53% kill; IC_{50}: 62.13µg/mL) compared to standard doxorubicin (95.90% kill; IC_{50}: 0.2µg/mL). Inhibitory Zone of the extract at concentrations (30, 60, 90µg/mL) against *Bacillus subtilis, Serratia marecescens, Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa, Strptotropomonas maltophilia, Escherichia coli, Salmonella typhi, and Saccharomyces cerevisiae, Candida albicans, Candida glabrata* was measured. Manifestation of intensified results against Gram-negative *Serratia marecescens* qualifies the *S. heteromalla* extract as a considerable source of narrow spectrum antibiotic. However, antifungal activity against *C. albicans* was found to be logical. Antioxidant potential was determined through DPPH assay which declared no notable antioxidant effects. To the best of our knowledge this is first research and report on above mentioned biological studies of *S. heteromalla.*

Keywords: *Saussurea heteromalla,* anti-anticancer, antioxidant, antibacterial, antifungal potential.

INTRODUCTION

Natural products are privileged resource initially as traditional herbal medicine which leads to the modern molecular drugs (Cragg and Newman, 2013). The ancient civilizations report confirmatory evidence encouraging use of natural products for treating vast range of diseases (Helmstädter and Staiger, 2014), 60% drugs in the market are natural product based, as in case of anticancer drugs (Lahlou, 2013). Genus *Saussurea* (Family: Asteraceae) is a rich source of medicinal species being used for several treatments both historically and current age herbal medicines with significant commercialization of these products. About 300 out of 400 species of genus *Saussurea* occur in South East Asia along with Pakistan and are used in local traditional herbal products to treat internal heat or fever, menstruation, unbalanced blood circulation, unwanted bleeding, body pain and rheumatic arthritis(Cheng *et al*., 2016).

Some of its commonly important plants are also used as ethnomedicine (Madhuri *et al*., 2012). *Saussurea heteromalla* (*S. heteromalla*) is bioactive natural product abundantly found in Islamabad and based upon the scientific principles it may be rich and important variety which is needed to be investigated for its therapeutic application especially as potential anti-cancer agents. Therefore crude extract of *S. heteromalla* indigenous to Islamabad, Pakistan is evaluated for cytotoxic, antioxidant, antibacterial and antifungal activity.

MATERIALS AND METHODS

Plant collection and extraction

Saussurea heteromalla plant was identified by Professor Dr. G.A Miana, collected from sector G-15 of Islamabad Pakistan during April and July 2017. Shade dried whole plant specimen was authenticated by Dr. Muhammad Zafar and deposited in department of medicinal plants herbarium Quaid-e-Azam University Islamabad with reference number (ISL-130608). After removing sand particles and intercalating debris by washing with water the whole plant (root, stem, leaves, flower and seeds) was dried in shade for four weeks, grinded to make fine powder and packed in air tight bag at room temperature. The fine powder was subjected to extraction procedure to obtain a crude extract, accurately weighed powdered (1.75Kg) plant material was placed in percolator for 2 weeks and soaked in 5 litres of methanol and methanolic extract was collected in 1000ml beakers. Percolation

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process was repeated and both the extracts were combined and concentrated through rotary vacuum evaporator (Buchi, Switzerland) to obtain final crude methanolic extract (Handa *et al*., 2008). The % yield of crude methanolic extract (CME) of *S. heteromalla* was found to be 63%, calculated by percent extract recovery by using the formula as follows:

$$\% \text{ Extract recovery } (\%w/w) = \frac{(A_{\text{dried extract weight}}}{B_{\text{powdered plant material weight}})} \times 100$$

Biological evaluation

Anticancer activity / Cytotoxicity assay

The in vitro cell toxicity profile of the *S. heteromalla* CME was investigated by performing 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay (Mosmann T *et al*., 1983) using NIH /3T3 and HeLa cell lines. MTT assay is used to screen for the cell proliferation, cytotoxic effects and viability (Radhakrishnan *et al*., 2001). IC_{50} values (concentration at which 50% of cells were viable) was determined. The assay was performed in HEJ Research Institute of Chemistry, Karachi.

Micro culture Tetrazolium (MTT) assay

Principle

Principally in MTT assay the enzyme Mitochondria succinate dehydrogenase reduces the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into colored and insoluble, Formazan substance which can be measured spectrophotometrically (Mosmann, 1983). However MTT reduction is only feasible in living and metabolically active cells, therefore this conversion can be related to the viable cells (living cells).

Procedure

Non cancer murine fibroblast cells (normal cells) NIH-3T3 and HeLa cell lines of human cervical cancer cells were cultured in 10% phosphate saline buffer containing Dulbecco's modified eagle medium (DMEM) supplemented with streptomycin and penicillin (each 50units /ml) in 5% CO2and 95% humid air at 37°C. NIH /3T3 and HeLa cells were seeded in 96 well microplates having density of 6 x 10^3 and 6 x 10^4 cells /well respectively in 200µl culture medium, the testing samples of S. *heteromalla* CME were dissolved in DMSO to prepare stock solution (10 mM), further diluted in medium to achieve concentration of 25, 50, 100 and 200µg /ml (concentration of DMSO 0.5%) after 24 h each concentration were added to the cells. Microplates were subjected to cultivation for 72 h at 37 °C, 5% CO_2 and 95% humidity, After incubation MTT solution (0.5mg /ml phosphate buffered saline, pH=7.4) was added to each well microplate followed by further incubation for 2hrs. After this media containing MTT solution was separated and from viable cells Formazan purple crystals were dissolved in lysis solution (0.1% Nonidet P40 in ethanol +4mM HCl). Absorbance was measured in microplate reader (EL x 808, BioTek, USA) at a wavelength of 570nm. Cells were incubated with only medium for achieving negative control. Each value is the mean of 6 wells with standard deviation. Doxorubicin was used as standard for HeLa cell lines, whereas cyclohexamide was used as standard for 3T3 cell line. The growth inhibitory effect of the extract was calculated by using the absorbance of test samples, blank, and negative control (without drug). For each compound IC_{50} (concentration of the extract or standard drugs inhibiting 50% growth of cells) was determined and calculated by Sigma Plot and Percentage toxicity (% cell inhibition) and Percentage cell viability (% cell survived) was measured by the formula given below:

$$\% \text{ cell inhibition} = 100 - \frac{(A_{\text{test compound absorbance}} - A_{\text{blank absorbance}})}{(A_{\text{control absorbance}} - A_{\text{blank absorbance}})} \times 100$$

Data interpretation

Less absorbance values compared to the control cells stipulate decline in the cell proliferation rate. On the other hand, more absorbance point toward raised cell proliferation rate.

$$\% \text{ cell survival} = \frac{(A_{\text{test compound absorbance}} - A_{\text{blank absorbance}})}{(A_{\text{control absorbance}} - A_{\text{blank absorbance}})} \times 100$$

% cell inhibition = 100 - cell survival

Anti Oxidant activity

The free radical scavenging activity was measured by using (2,2-diphenyl-1-picryl-hydrazyl) DPPH assay (Obied *et al*., 2005) DPPH (3.2mg) was dissolved in 100ml mixture of methanol and water (82:18). Solution of Ascorbic Acid & Resveratrol (references) and *S. heteromalla* CME (test sample) was prepared separately in the concentration range of 62.5-1000 µl/ml. (2800µl) solution of DPPH was added to each glass vial followed by 200µl addition of each concentration of reference solutions and sample solutions respectively. After shaking well, Mixtures were incubated in dark at temperature 25 ± 2°C for one hour. Absorbance was recorded at 517nm through UV-Visible Spectrophotometer (Shimadzu 1800). Ascorbic acid and Resveratrol were considered as positive control. The test was repeated as triplicate.

% inhibition of the DPPH radical by the all test samples was determined according to below mentioned formula and IC_{50} value was calculated by IC_{50} calculator software

$$\text{Scavenging Effect } (\%) = \frac{(A_{\text{negative control absorbance}} - A_{\text{test sample absorbance}}}{A_{\text{negative control absorbance}})} \times 100$$

Antimicrobial activity

S. heteromalla CME was subjected to screening for antibacterial and antifungal potential against 8 different G-negative and G-positive human pathogenic bacterial strains like *Salmonella typhi* ATCC 14028*, Escherichia coli* ATCC 8739*, Pseudomonas aeruginosa* ATCC 9027*, Strptotropomonas maltophilia* ATCC 13637*, Serratia marecescens* ATCC 13880 (Gram-negative) and G-positive *Micrococcus luteus* ATCC 9341*s, Bacillus subtilis* ATCC 5230*, Staphylococcus aureus* ATCC 6538 *and* three fungal strains like *Saccharomyces cerevisiae* ATCC 9763*, Candida glabrata* ATCC 90030 and *Candida albicans* ATCC 10261 respectively by performing disc diffusion assay (Bauer *et al*., 1966). The strains of bacteria and fungi were obtained from Amson vaccines & Pharma quality control laboratory Islamabad, microbiology laboratory of Islamia University Bahawalpur, Bahawalpur and Riphah Institute of Pharmaceutical Sciences, Riphah International University, Islamabad.

Preparation of Gemifloxacin, Nystatin and S. heteromalla CME Solutions

Gemifloxacin mesylate (18.7 mg), Nystatin (15mg) and *S. heteromalla* CME (15mg) was dissolved separately in 50 ml of methanol to prepare Gemifloxacin, Nystatin and *S. heteromalla* CME stock solutions. From these stock solutions 2.5, 5.0 and 7.5 ml was transferred to 25 ml volumetric flasks separately and make up volume with methanol to obtain 30µg, 60µg and 90µg per ml concentration of Gemifloxacin, Nystatin and *S. heteromalla* CME respectively.

Preparation of microbial culture

Nutrient broth (10 ml aliquots) was inoculated with the test bacterial strain separately and incubated for 24 hours at 37°C, fungal strains were cultured in Sabouraud Dextrose agar (SDA) 0.13g of Sabouraud dextrose broth (SDB) powder was dissolved in 10ml distilled water, Three fungal strains were cultured in SDB separately in a sterile test tub and incubated at 28°C until used for antifungal activity. Microbial culture density to conduct the antibacterial and anti fungal tests were maintained at 0.5 McFarland standard with the Turbidometer (Oxoid, UK).

Nutrient agar medium for bacterial growth and Sabouraud dextrose agar (0.62g of SDA powder was dissolved in 1000 ml of distilled water, pH was adjusted to 5.6±0.2) medium for fungal growth were also prepared, autoclaved, mixed well, cooled to 45°C and 25ml was added separately under aseptic conditions into a sterile Petri dish, each Petri dish was again incubated at 37°C for 24 hours to check any microbial growth in the agar medium. Microorganism cultures were inoculated with the help of sterile cotton swab on each petri plate .Triplicate plates of each test organism was prepared.

Disc diffusion method

The In vitro each concentration of test crude sample (30µg, 60µg and 90µg/ml) against test organism was evaluated through disc diffusion technique. 4mm filter paper discs of Whatman No.1 were loaded with above concentrations of test samples. Each disk was impregnated with specified concentration of the extract by first applying 10µl with the pipette, left to evaporate, then applying another 10µl, and then drying again and so on. The positive control comprises the standard antibiotic Gemifloxacin mesylate disc in case of antibacterial action while against fungal strains Nystatin was used as reference substance. Negative controls contained sterile disc only (DMSO) to carry out both activities. Using sterile forceps, the test samples and reference drugs loaded discs were kept on the agar plates previously inoculated with test microbial cultures. Seven days old fungal culture was used. Then the agar plates were kept for incubation at room temperature (27°C±2) for 24h, after incubation plates were examined for zones of inhibition that were measured in millimeters. The test was performed in triplicate to ensure reliability. The results are shown in table 3.

STATISTICAL ANALYSIS

The results obtained from cytotoxic, antioxidant and antimicrobial assays were expressed as mean ± SD from three times repeated analyses, also were analyzed statistically by one-way analysis of variance (ANOVA) by applying PASW Statistical Package 18.

RESULTS

Anticancer / cytotoxic activity

Table 1 shows the cytotoxic effects of *S. heteromalla* CME against HeLa cell line and NIH / 3T3 cells using MTT Assay. After 48 h of exposure, the plant extract demonstrated strong percent inhibition (28.63±1.69, 45.17 ±0.85, 61.17±0.85, 77.28±1.53) against HeLa cells growth at varying efficacies in a concentration dependent manner at all tested concentrations 25, 50, 100, 200 µg/ml respectively with IC_{50} value 62.13µg/ml compared with the cytotoxicity of reference doxorubicin that manifested percentage inhibition 95.90; IC_{50} value 0.2±0.03 µg/ml, While the growth of NIH / 3T3 cells was moderately inhibited with IC_{50}; 68.4µg/ml in contrast to the HeLa cells. Cyclohexamide standard for NIH / 3T3 cell line displayed IC_{50}; 25.48µg/ml, no significant difference ($P<0.03$) was found in all of samples (table 1).

Antioxidant activity

Among various methods utilized to determine the antioxidant activity of biological compounds DPPH assay was employed to determine the antioxidant potential of *S. heteromalla* CME at 62.5, 125,250, 500, and 1000µg/ml. The % free radical scavenging activity (% FRSA) of *S.*

heteromalla CME was measured and IC_{50} value was calculated. Ascorbic acid and Resveratrol were used as reference standards. The *S. heteromalla* CME did not manifest any radical scavenging effect at any concentration tested as its IC_{50} was >1000µg/ml compared to Ascorbic acid and Resveratrol reference standards IC_{50}; < 62.5 and 141.74µg/ml respectively there significant difference in the samples was ($P < 0.05$) (table 2).

Antibacterial activities

The susceptibility of 8 bacterial strains towards *S. heteromalla* CME was evaluated by measuring the size of inhibition zone in millimeters at three different concentrations 30, 60 and 90 µg/ml. DMSO was used as negative control to rule out antibacterial activity therefore it displayed no inhibition. The size of zone of inhibition depends upon the concentration of the *Saussurea heteromalla* crude extract (fig. 2). *Escherichia coli* (11.5-13.57mm), *Pseudomonas aeruginosa* (11.2-13.13mm), *Salmonella typhi* (10.95-12.8mm), *Strptotropomonas maltophilia* (8.67-15.23mm), *Serratia marcescens* (13.87-20.65mm), *Bacillus subtilis* (6.45-7.83mm), *Staphylococcus aureus* (6.78-9.78mm) and *Micrococcus luteus* (9.57-10.92mm), there were no significant difference ($P<0.05$) in all samples (table 3).

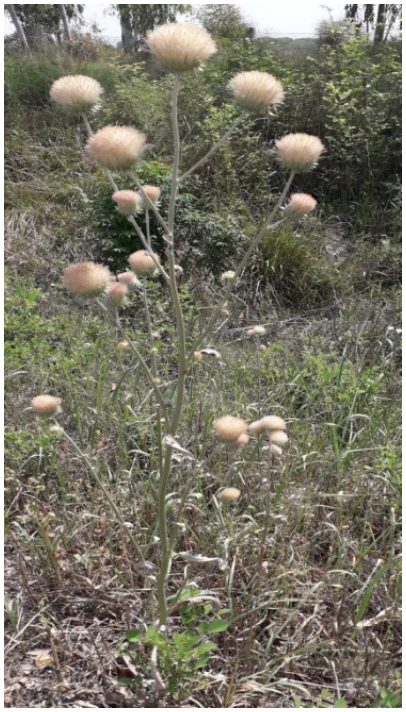

Fig. 1: *S. heteromalla* (Islamabad, Pakistan)

Antifungal activities

The in vitro antifungal activity of the *S. heteromalla* CME against three fungal strains employed and its susceptibility potential was predicted by the presence or absence of zones of inhibition at three different concentrations 30, 60 and 90 µg/ml compared to the standard antifungal drug Nystatin (table 3). The extract exhibited moderate inhibitory activity against *Candida albicans* (8.22-9.26mm), whereas no inhibitory action was seen against *Candida glabrata* and *Saccharomyces cerevisiae*.

Table 1: Percent Inhibition and IC_{50} of *S. heteromalla* CME, Cyclohexamide and Doxorubicin against HeLa and NIH/3T3 Cell lines.

Concentration (µg/mL)	Percent (%) Inhibition	
	NIH 3T3	HeLa Cell Line
25	23.91±0.28	28.63±1.69
50	41.56 ±1.37	45.17 ±0.85
100	59.54 ±1.64	61.17 ±2.45
200	73.88 ±1.96	77.28 ±1.53
IC_{50} (µg/mL)		
S. heteromalla	68.4	62.13
Cyclohexamide	25.48	—
Doxorubicin	—	0.2±0.03

Values are presented as mean ± Standard deviation (n = 3).

Table 2: antioxidant Activity of *S. heteromalla* CME, Ascorbic acid and Resveratrol

DPPH Radical Scavenging Activity			
Concentration (µg/ml)	Percent (%) Inhibition		
	Ascorbic acid	Resveratrol	*S. heteromalla*
1000	NA	NA	31.45
500	96.73	95.84	19.44
250	97.33	59.68	12.61
125	97.18	51.82	9.05
62.5	97.03	35.05	6.08
IC_{50} (µg/ml)	< 62.5	141.74	> 1000

Values are presented as mean ± Standard deviation (n = 3).

DISCUSSION

Present investigation explored the cytotoxic, antioxidant, antibacterial and antifungal potential of *S. heteromalla* CME and is the first report on afore mentioned effects of *S. heteromalla* CME as well. Previous study stated that potent anticancer agent derived from plant must have IC_{50} value less than 100 µg/mL, otherwise it cannot to be developed as a chemoprotective agent (Prayong *et al*., 2008). The plant extract demonstrated strong cytotoxic potential against HeLa cells growth with different efficacies at each concentration with IC_{50} ; 62.13 µg/ml compared with the cytotoxicity of standard doxorubicin IC_{50}; 0.2±0.03 µg/ml, While the growth of NIH / 3T3 normal cells was moderately inhibited with IC_{50}; 68.4µg/ml as compared to the HeLa cancer cells. Although cytotoxicity of *S. heteromalla* CME against HeLa cell line is potent at 200µg/mL but this observed inhibitory effect is lower as compared to standard doxorubicin. Induction of cytotoxicity by *S. heteromalla* CME may be attributed to high phenolic content,

Table 3: Zone of Inhibition of *S. heteromalla* CME, Gemifloxacin Mesylate and Nystatin, Values are presented as mean ± Standard deviation (n = 3)

Antibacterial Activity (Zone of Inhibition (mm)						
	Gemifloxacin			*S. heteromalla*		
Concentration (µg/ml)	30	60	90	30	60	90
Escherichia coli	35.7	35.97	40.78	11.5	12.27	13.57
Pseudomonas aeruginosa	14.4	23.56	33.95	11.75	13.13	11.29
Salmonella typhi	27.51	30.54	32.55	10.95	12.67	12.87
Strptotropomonas maltophilia	20.14	30.45	41.46	8.67	9.11	15.23
Serratia marcescens	22.35	22.89	45.31	13.87	16.2	20.65
Bacillus subtilis	14.33	18.56	25.92	6.45	6.96	7.83
Staphylococcus aureus	13.67	18.22	26.34	6.78	7.21	9.78
Micrococcus luteus	20.91	32.65	44.22	9.57	9.75	10.92
Antifungal Activity Zone of Inhibition (mm)						
	Nystatin			*S. heteromalla*		
Concentration (µg/ml)	30	60	90	30	60	90
Saccharomyces cerevisiae	16.51	17.63	19.06	—	—	—
Candida albicans	18.28	18.63	19.22	8.22	8.76	9.26
Candida glabrata	14.34	14.91	15.65	—	—	—

especially flavonoids and biologically active natural compound "Arctigenin" (phenyl propanoid dibenzyl butyrolactone lignin), previously confirmed and reported in the literature by the phytochemical analysis of *Saussurea heteromalla* (Saklani *et al*., 2011), Moreover flavonoids and Arctigenin exhibit various pharmacological actions including anticancer and antioxidant activities (Kou *et al*., 2011), Phenolics isolated from L. chinensis palm fruits are reported to exert potent anti-proliferative, antioxidant, and apoptosis against HeLa cancerous cells (Cheng *et al*., 2016), therefore being rich in flavonoids and Arctigenin *S. heteromalla* can be a valuable source of natural moieties to be used as new anticancer agent. Anticancer ability of polyphenols is affected by various factors including cancer type, chemical structure and working dose (Sak, 2014).

Literature Studies demonstrate a significant function of antioxidant mechanism in many anti-diabetic, anti-inflammatory, anticonvulsant, antiarthritic, heptoprotective, neuroprotective, antiulcer, cardio protective and wound healing drugs (Patil *et al*., 2012). DPPH scavenging assay was used to observe the antioxidant activity of *S. heteromalla* CME. DPPH being a stable free radical becomes a stable molecule by accommodating an electron (Rice-Evans and Miller, 1996). The antioxidants exert radical scavenging action due to having ability to donate hydrogen, In present research *S. heteromalla* CME did not exhibit antioxidant potential at any concentration tested as its IC_{50} was > 1000µg/ml compared to reference standards despite the presence of flavonoids and Arctigenin (antioxidants) previously investigated and reported in the literature by the phytochemical studies of *S. heteromalla* (Saklani *et al*., 2011). While investigating hydroxyl flavones consisting of one, two or three hydroxyl groups (Park *et al*., 2009) demonstrated that monohydroxy flavones do not exhibit strong free radical scavenging ability, whereas numerous dihydroxy and trihydroxy flavones display relatively high activity, even greater than vitamin C, This supports the claim that antioxidant activity of hydroxy flavones can be determined by the number and location of hydroxyl groups. Previous researcher established that the free radical scavenging capacity of 3', 6, 7-trihydroxyflavone was 87.8%. By the above mentioned claim it can be emphasized that *S. heteromalla* CME may be flavonoids rich entity with one hydroxyl group therefore it lacks any free radical binding capacity or antioxidant potential.

The development of new molecular lead compounds is indispensable as the conventional synthetic compounds have less ability and are unsatisfactory in combating the major infections caused by bacteria (NAIR *et al*., 2005), Therefore traditional plants are being increasingly investigated in search of new lead molecules to develop drugs with antimicrobial potentials (Sukanya *et al*., 2009). Although both the Gram-negative and positive bacteria were moderately inhibited by *S. heteromalla* CME at all concentrations but the G-negative organisms were more susceptible to the antibacterial action of *S. heteromalla*. This variation is probable due to the difference in the bacterial strains cell wall structure.

Among Gram-negative group *S. heteromalla* exhibited the maximum antibacterial activity against *Serratia marcescens* with zone of inhibition 13.87mm, 16.20mm and 20.65mm at all three concentrations tested respectively compared to the zone of inhibition established by standard drug Gemifloxacin 22.35, 22.89 and 45.31mm at all three concentrations tested respectively.

Against Gram-positive bacteria, *Micrococcus luteus* was more susceptible to the antibacterial action of *S. heteromalla* CME with zone of inhibition 9.57mm, 9.75mm and 10.92mm at all three concentrations tested respectively. Literature review based on antibacterial potential of phenolics revealed these phytochemicals as predominant source liable for activity against the G - positive bacteria (Rios and Recio, 2005). Further investigations also explored the same action produced by tannins and flavonoids (Ahmad and Beg, 2001, Madureira *et al*., 2012). It is accomplished that *Saussurea heteromalla* is enriched source of afore stated chemicals as previously reported in literature through phytochemical analysis therefore it could be a useful natural entities source to be used as new antibacterial agent (Puri *et al*., 2017), Comparison of antibacterial activity data (table 3) suggests that *S. heteromalla* CME is moderately active against almost all tested bacterial strains. Manifestation of intensified results against Gram-negative *Serratia marcescens* qualifies the *S. heteromalla* CME as a considerable basis of narrow spectrum antibiotic. Narrow spectrum medicines based monotherapy offers targeted access with the advantage of valuable platform to terminate the emergence of resistance generally related to broad spectrum antibiotics (Rea *et al*., 2011).

Candida albicans exist commensally and is a superficial contaminant that can elicit serious infections in humans (Kim *et al*., 2009), while *Candida glabrata* is an opportunistic yeast pathogen increasing in growth at alarming rate over the years, Regardless of its genus, its species correlates with *Saccharomyces cerevisiae* than *Candida* species (Gabaldón and Fairhead, 2019). *Saccharomyces cerevisiae* is common budding yeast investigated for effects of drugs against yeast strains. The occurrence of critical mycoses produced by opportunistic fungi is rising with immuno-deficiency patient's number including cancer, AIDS and organ transplants. Limited systemic antifungal drugs (flucytosine, amphotericin B, ketoconazole, itraconazole, miconazole) are effective to treat such mycoses but these drug therapies are unsatisfactory regarding effectiveness, antifungal spectrum, toxicity and drug resistance. Plants and their derivatives are well-known origin in biological inquiries having pronounced potential of biologically active constituents employed in drug development, hence it is necessary to develop new drug therapies consisting of natural products to alleviate *C. albicans* infections and other related fungal strains (St Georgiev, 1992). The *S. heteromalla* CME exhibited most prominent activity for *Candida albicans* with zone of inhibition 8.22, 8.76 and 9.26mm at 30, 60 and 90 µg/ml respectively, therefore can be effective against diseases like candidiasis. In contrast to the *Candida albicans, S. heteromalla* crude extract was completely ineffective against fungal strains of *Candida glabrata* and *Saccharomyces cerevisiae* at all the three concentrations tested as no zone of inhibitions for both species are present in the table 3 therefore, *S. heteromalla* CME cannot be considered as potential candidate to treat vaginal candidiasis caused by *Saccharomyces cerevisiae*.

The antifungal activity *of S. heteromalla* CME against *Candida albicans* may be associated with the existence of flavonoids, phenolic, essential oils and tannins (Cowan, 1999). Flavonoids and Tannins have the potential to disrupt the integrity of fungal cell wall by making complexes with extracellular proteins therefore well intended an antifungal agent (Arif *et al*., 2009), Hence antifungal activity manifested by the *Saussurea heteromalla* crude extract can be endorsed by flavonoids, tannins and phenolic compounds whose occurrence is already confirmed and reported by phytochemical investigations of plant (Puri *et al*., 2017)**.** Despite the presence of aforesaid phytochemicals *S. heteromalla* CME have no activity against *Candida glabrata* and *Saccharomyces cerevisiae*; Investigational studies are required to explore the diversified antifungal behavior of *S. heteromalla* against different fungal strains.

CONCLUSION

Our investigational studies postulate crude methanolic extract of *S. heteromalla* as a potentially enriched source of cytotoxic lead compound for phytomedicine with high-ranking MTT solution reducing properties and a striking antibacterial activity against gram-negative Serratia *marecescens and* gram-positive bacterial strain *Micrococcus luteus* compared with all other tested strains, *S. heteromalla* in this regards deserves further scientific investigations based on isolation and characterization to proclaim the biologically active lead molecules demonstrating the evident potential.

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