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EVALUATION OF ANTIOXIDANT, ANTI-CANCER AND ANTIMICROBIAL ACTIVITY OF DIFFERENT EXTRACTS AND FRACTIONS OF *STEPHANIA GLABRA*

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ABSTRACT

Stephania glabra is a wild medicinal plant possessing multiple uses as anti-cancer, antimicrobial and antioxidant activities. Different extracts and fractions were prepared from *S. glabra* tubers and were analyzed for different activities. Evaluation of the total phenolic and flavonoid content revealed that chloroform extract of *S. glabra* contain maximum phenolic content and maximum flavonoid content was found in methanolic extract. Dry powder analysis of the tubers revealed the presence of saponins, alkaloids and proteins. Ethyl acetate extract of *S. glabra* tubers showed highest antioxidant potential as revealed by ABTS radical scavenging activity. Methanolic extract has the highest anti-cancer activity while chloroform extracts and fractions have highest antimicrobial activity. These activities can be attributed to the high amount of the phytoconstituents present in these extracts

Keywords: Anti-cancer; antimicrobial; antioxidant; growth curve; phytochemicals

INTRODUCTION

Traditional uses of medicinal plants in the form of powder, liquid or mixtures include as treatment against various ailments in India. These herbal plants have proved pivotal in the development of new drugs or phytomedicines for treatment of diseases. Many plants with medicinal properties have been reported from India (Chopra *et al.,* 1958; Dhar *et al.,* 1965, 1968; Dhawan *et al*., 1980; Rao and Krishnaiah, 1981; Bhakuni *et al.,* 2002; Shilaskar and Parasher, 1989). Several plant species (medicinal or non-medicinal) have been used for their remedial properties for centuries against human diseases as these contain components of therapeutic value (Iwu *et al.,* 1999). During the last 20 years, various plant parts such as leaves, roots, shoot, rhizomes, etc. or their extracts have been used extensively as complementary and alternative medicine (Rios and Recio, 2005). Antimicrobial potential of different medicinal plants is also studied extensively worldwide (Kaur and Arora, 2009). Diseases like cancer, jaundice and asthma have been treated with the plant rhizomes and tuberous roots in indigenous systems of medicines in Asia especially in India, Pakistan and China (Asif, 2012). The rhizomes are the horizontal underground plant stem capable of producing the shoot and root systems of a new plant. The capability of rhizome to produce shoots and roots, allows the parent plant to propagate vegetative and to perennate underground (Auta *et al.,* 2011).

The interest of pharmaceutical industries has increased to discover the new drugs from the ethnobotanicals and also to provide new and alternative methods to synthetic drugs for the treatment of dreadful diseases. *Stephania glabra* belongs to Menispermaceae family, which includes 65 genera and 350 species (Semwal *et al.,* 2010, Hong *et al.,* 2015, Moongkarndi *et al.,* 2004, Nakaoji *et al.,* 1997, Montrirattigri *et al.,* 2008 and Yang *et al.,* 2010). *S. glabra* (*Roxb*.) Miers, vern., Gindaru is one of the well-known members of this genus. The plants belonging to this family (especially tubers) have wide range of medicinal properties and they have been used traditionally by the locals for the treatment of dysentery, hyperglycemia, asthma, tuberculosis, cancer, fever, intestinal complaints, sleep disturbances and inflammation (Kirtikar and Basu., 2004, Gaur., 1999 and Chopra *et al.,* 1958). *S. glabra* is a large, climbing shrub, indigenous to the lower parts of Indian Himalayan region (Gaur 1999). Large number of phytochemical studies on this plant has led to the isolation of over 30 alkaloids with a very few flavonoids, and other constituents (Bhakuni & Gupta 1982; Pal *et al*., 1995). The present communication deals with the analysis of antioxidant, anti-cancer and antimicrobial activities of *S. glabra* tuber extracts and fractions.

MATERIALS AND METHODS

Reagents

All the reagents and the solvents used in the study were of analytical grade. 2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid (ABTS), Dulbecco's modifies eagle (DMEM) high glucose media, fetal bovine serum (FBS), resazurin dye (7-hydroxy-10-oxidophenoxazin-10-ium-3-one, sodium), agar, nutrient b-roth were purchased from HiMedia, Mumbai, India. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was purchased from Thermo fischer Scientific.

Preparation of plant powder

The plant material consisted of tuber of *Stephania glabra* harvested in the month June, 2017 in the Neri village of solan district, himachal Pradesh corresponding to the geographical coordinates: 30.9086989 ° N, 77.10042 ° E. The plant was authenticated at Dr. Y.S. Parmar University of Horticulture & Forestry Nauni-Solan and voucher specimens was deposited in the herbarium and were entered in the UHF-Herbarium with Field Book No. 1181, receipt no. 096. The tuber was washed and the external peel was removed. It was chopped, washed and dried for few days till no moisture was left. The dried tuber was grinded and the coarse powder was passed through sieve #35 (0.5mm) to obtain powder with smaller particle size.

Preparation of plant extract

S. glabra extracts were prepared after defatting the powder by suspending it in petroleum ether in the ratio 1:5 for a day. Methanolic extract was prepared by suspending the powder in the respective solvent in the ratio 1:10 for 3 days. This was done by cold maceration (30 °C 150 rpm) and Soxhlet (60 °C) and the yield of the extract was compared for effectiveness of extraction. The suspension was filtered through whattman filters (grade 1-125mm) and the solvent was allowed to evaporate. The dried methanol extract was re-suspended in 100ml de-ionized water and was fractionated based on polarity by the liquid-liquid fractionation using petroleum ether, chloroform, ethyl acetate and butanol, respectively. Other extracts were prepared by suspending powder in the respective solvent in the ration 1:10 for 3 days by cold maceration at 30 °C 150 rpm. Each extract and fraction was dried by vacuum evaporation and the yield was calculated as follows:

$$\%\,Yield = \frac{Weight of the extract or fraction recovered}{Weight of the powder used} \times 100$$

Phytochemical Screening

Phytochemical screening tests were used to characterize the presence of secondary metabolites based on solubility tests, color reactions and precipitation. The phytochemical screening was done by following the standard protocols (Shaik *et al.,* 2011, Vimalkumar *et al.,* 2014).

Determination of Total Phenolic Content

A volume of 0.01 ml of the miscella was introduced into 96 well plates followed by 0.05 ml Folin-Ciocalteu's reagent (diluted 10 times with water). The solution was then kept in the dark for 5 min and then 0.04 ml sodium carbonate (7.5 % w/v) was added. After 30min, absorbance of the mixture was measured by Varioskan LUX, Thermo Scientific light absorption spectrophotometer at 765 nm and compared to a gallic acid calibration curve. Gallic acid served as the standard for preparing the calibration curve ranging from 0.10 to 1 mg/ml assay solution. The determinations were carried out in triplicate and the total phenolic content was expressed as Gallic acid equivalents (mg of GAE/g of sample) (Pourmorad *et al.,* 2006).

Determination of Total Flavonoid Content

An aliquot (0.1 ml) of the sample was mixed with 0.01 ml of 5 % sodium nitrite. After 5 min, 0.01 ml of 10 % aluminum chloride was added to the mixture and mixed. After 6 min, 0.1 ml of 1M sodium hydroxide was added to the mixture. Absorbance of the reaction mixture was measured at 510 nm against a blank by Varioskan LUX, Thermo Scientific light absorption spectrophotometer. The flavonoid content was determined using a standard curve of ascorbic acid. Ascorbic acid served as the standard for preparing the calibration curve ranging from 0 to 10 mg/ml assay solution. The determinations were carried out in triplicate and the total phenolic content was expressed as Ascorbic acid equivalents (mg of AA/g of sample) (Zhishen *et al.,* 1999, Rebaya *et al.,* 2014).

Dry Powder Analysis

The dry powder of *S. glabra* was analyzed for the alkaloid content as described by Harborne with slight modifications. The residue formed was the alkaloid content which was vacuum dried and expressed as mg/g of the powder. Saponin content was analyzed as described by Mir *et al.*. The extract obtained was the saponin content which was vacuum dried and expressed as mg/g of powder (Nahapetian and Bassiri, 1974 and Mir *et al.,* 2016). Protein content was analyzed as explained by Dawes *et al.*. The protein content was determined using a standard curve of bovine serum albumin (BSA). BSA served as the standard for preparing the calibration curve ranging from 1 to 10 mg/ml assay solution. The determinations were carried out in triplicate and the protein content was expressed as mg protein/g of powder (Dawes *et al.,* 1974 and Lowry *et al.,* 1951).

***In vitro* antioxidant assay**

ABTS assay was conducted to analyze antioxidant attributes of *S. glabra* extracts and fractions (Sharma *et al.,* 2018). ABTS solution was prepared by dissolving ABTS and ammonium persulfate in distilled water to get final conc. of 7.4 mM ABTS: 2.46 mM ammonium persulfate. The solution was kept in dark for 12-16 h. Further the working solution was prepared by diluting the solution

1:25 in methanol to get a final absorbance of 0.7 ± 0.05 at 734 nm. Sample extract was prepared in DMSO (1 mg/ml). Ascorbic acid and ABTS solution in methanol were considered as positive control and methanol was used as a blank whereas DMSO was used as negative control. To 1 ml of $ABTS^{\bullet+}$ solution, 10 µl sample extracts containing antioxidant were added at the conc. 20, 40, 60, 80 and 100 µg/ml. Absorption was taken using Varioskan LUX, Thermo Scientific light absorption spectrophotometer at a wavelength of 734 nm. The assay was performed in triplicate three times and percentage inhibition was calculated using the following formula:

$$\%\ Inhibition = \frac{Abspositivecontrol - AbsTest}{AbsControl} \times 100$$

And IC_{50} value was calculated by Graph pad Prism 5.0.2.

Cell culturing and maintenance

To evaluate anticancer properties of *S. glabra,* A549 (human lung adenocarcinoma), PC-3 (human prostate carcinoma) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. The cells were cultured in DMEM supplemented with 10% inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin solution (10,000 Units/ml penicillin and 10,000 µg/ml streptomycin) in a humidified atmosphere of 5% CO_2 at 37° C until confluent.

***In vitro* cytotoxicity assay**

The cells (5 × 10 cells/well)
were cultured in 96-well microtiter plate followed by treatment with varying doses of extract and fractions (25, 50, 100, 200 µg/ml) for 24 h. Cells were washed twice with PBS after treatment and then incubated with 20 µl of MTT (5 mg/ml) in fresh medium for 4 h at 37 °C, followed by solubilization of the formazan crystals by DMSO. The absorbance of the individual sample was measured using a microplate absorbance reader (Varioskan LUX, Thermo Scientific light absorption spectrophotometer) at 570 nm. Percentage of cell death was computed using the following equation:

$$\%\ CellDeath = \frac{O.D.Control - O.D.Sample}{O.D.Control} \times 100$$

Where OD control is an optical density for untreated cells, and the OD sample is an optical density for cells treated with extracts and fractions (Kumari *et al.,* 2020). Untreated cells were used as negative control and vincristine (4 µg/ml) treated cells were used as a positive control. IC_{50} values were calculated by Graphpad Prism 5.0.2.

Anti-Microbial Assay

The antimicrobial activity of extracts and fractions was checked against both Gram +ve bacteria such as *Bacillus subtilis* and Gram −ve bacteria like *Pseudomonas aeruginosa*. Minimal inhibitory concentrations (MICs) were determined by resazurin assay by using 96-well microtiter plate. Different conc. of extracts and fractions ranging from 100 mg/ml to 0.39 mg/ml was maintained in the 96-microtiter plate for 24 h at 37 °C. After 24 h resazurin was added to a final conc. of 60 µM and was further incubated for 2-4 h for the observation of color change. On completion of the incubation, columns with no color change (blue resazurin color remained unchanged) were scored as above the MIC value. The mean of live cells was recorded using Varioskan LUX, Thermo Scientific light absorption spectrophotometer. Since, MIC is the lowest conc. at which no visible growth of the test pathogens was observed, therefore the value at which OD_{600} was zero was selected as MIC for the specific extract/fraction (Semwal *et al.,* 2009).

Colony Forming Unit (CFU)

10^7 colony-forming units (CFU) of bacterial cultures were cultured on LB agar plates supplemented with extracts and fractions at 12.5 µg/ml (Sondi and Salopek-Sondi, 2004). Extract and fraction free LB plates and ampicillin LB plate were cultured under the same conditions and were used as controls. The plates were incubated for 24 h at 37 °C and the numbers of colonies were counted. The counts on the three plates corresponding to a particular sample were averaged and expressed as percentage inhibition using the following formula:

$$\%\ Inhibition = \frac{(No.ofcoloniesofuntreatedbacteria - No.ofcoloniesoftreatedbacteria)}{No.ofcoloniesofuntreatedbacteria} \times 100$$

Statistical analysis

Data are represented as mean ± SEM and statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using Graph Pad Prism 5.0. Also, a p value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Phytochemical screening

Various methods were used to qualitatively analyze the presence of different phytochemicals in isolated extracts and fractions. The data revealed that *S. glabra* tubers were found to contain alkaloids, quinones, carbohydrates, phenols and proteins in abundant quantity whereas tannins, saponins, resins, cumarins, flavonoids were found in limited quantity as depicted in supplementary table 1 and 2. Earlier, also it has been shown that ethanolic extract of *S. glabra* showed the presence of alkaloids, phenols, cardiac glycosides, tannins, saponins and steroids whereas, flavonoids were absent. The total phenolic content of *S. glabra* was found to be 286.12 ± 3.14 mg/g of gallic acid equivalent (GAE) (Singh *et*

al., 2018). It was also found that different methods of phytochemicals screening do not make a difference to the phytochemicals largely (Semwal and Semwal, 2015). *S. glabra* is an alkaloid rich plant which has more than 30 alkaloids (Bhakuni and Gupta, 1982; Semwal and Semwal, 2015; Patra *et al.,* 1980). The extraction efficiency varies significantly with different extraction methods applied on same plant material with the same solvent system. Apart from a compatible extraction method the choice of appropriate solvent system is of prime importance. Like dissolves like principle is applicable for the selection of appropriate solvent system as polar solvents extract out polar compounds and non-polar compounds will be extracted out by non-polar solvents (Gupta *et al.,* 2012).

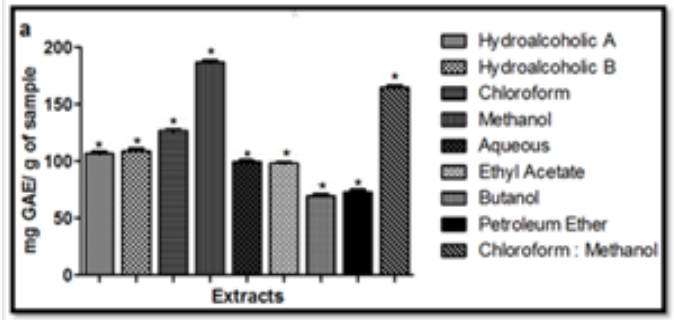
Dry Powder Analysis

The plant tuber powder was analysis determined 96 mg alkaloid/ g of the tuber powder. Saponin content was found to be 7.5 mg saponin/ g of the tuber powder whereas; protein content was found to be 44.10 mg protein/g of the tuber powder. The methanol and aqueous extracts of *Crocus sativa* (saffron) were found to contain 1.2 and 3.4 mg/g of saponin content, 6.4 and 2.4 mg/g of alkaloid content, respectively (Mir *et al.,* 2016).

Total Phenolic Content

The total phenolic content was reported as Gallic acid equivalents with reference to a standard curve ($y = 0.6375x + 0.124$, $R^2 = 0.9929$). The analysis revealed that methanolic extract showed maximum phenolic content followed by chloroform: methanolic extract (Fig. 1a). The phenolic content in different fractions was lesser as compared to the methanolic extract (Fig. 1b). Similarly, total phenolic content of *S. glabra* ethanolic extract was found to be 286.12 ± 3.14 mg/g of GAE (Singh *et al.,* 2018).

The difference in phenolic content is due to the extraction of compounds of different polarity in different solvent systems. The solubility of different compounds depends on the polarity of the solvent system used for extraction and the chemical nature of the plant sample. Phenolics are present in plant sample either in simple form, highly polymerized form or in a combination with other plant components such as carbohydrates and proteins (Dai and Mumper, 2010, Xu and Chang, 2007). Methanol is generally found to be more efficient in extraction of low molecular weight polyphenols while high molecular weight flavanols are extracted better with aqueous



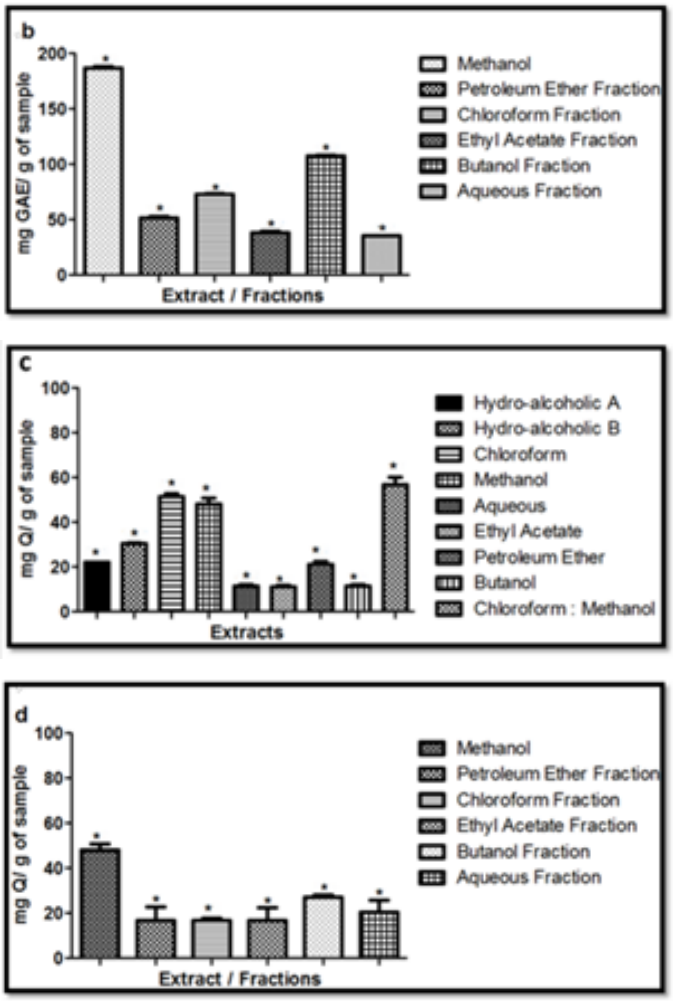


Fig 1: Total phenolic content of extracts (a) and fractions (b) expressed as mg GAE/ g of sample. Total Flavonoid content of extracts (c) and fractions (d) expressed as mg Q/ g ofsample. Results represented as mean ± SEM of three different experiments. Data is statistically significant with *p < 0.05 when compared with control.

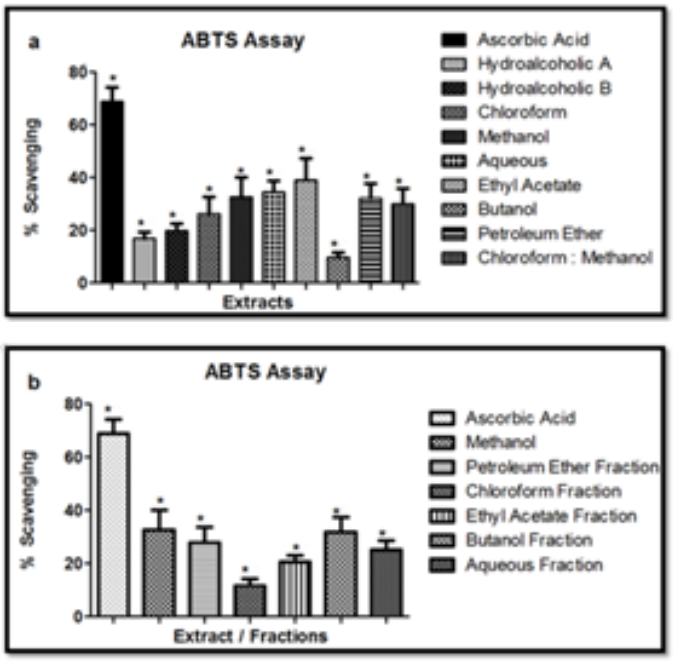


Fig 2: ABTS % radical scavenging of extracts (a) and fractions Results represented as mean ± SEM of three different experiments. Data is statistically significant with *p < 0.05 when compared with control.

TEST	Methanolic Extract	H_2O Control	Petroleum ether fraction	Chloroform fraction	Ethyl Acetate fraction	Butanol fraction	Aqueous fraction
ALKALOIDS							
Dragendorff	+++	-	+	++	+	++	+++
Mayer's Test	+++	-	++	+++	++	+++	+++
FLAVONOIDS							
$FeCl_2$	-	-	+	+++	++	++	-
Alkaline Reagent	+	-	+	++	-	+	-
Shinoda	+	-	-	-	-	+	+
Lead Acetate	++	-	+	++	+	++	+++
CARBOHYDRATES							
Molisch's Test	+++	-	+++	+	++	+++	+++
Benedict's							
Fehling's	+++	-	+	++	-	+	+++
	++	-	++	+	-	+++	++
PHENOLS							
$FeCl_2$	-	-	+	+++	++	++	-
Lead Acetate	++	-	+	++	+	++	+++
Iodine	++	-	+++	+++	+	++	++
PROTEINS							
Ninhydrin	-	-	-	-	-	-	-
Xanthoproteic	+++	-	++	++	+	+++	++
Biuret	++	-	+	++	-	+	+
SAPONINS							
Foam Test	+++	-	+	-	-	+++	-
Froth Test	+++	-	+++	-	-	+++	-
GLYCOSIDES							
Keller Killiani	-	-	-	-	-	-	-
Borntrager	-	-	-	-	-	-	-
Legal's Test	+++	-	++	++	+	+++	+++
Anthraquinone glycosides	++	-	-	-	-	+++	+++
TANNINS							
Gelatin	-	-	+	++	+	+	++
$FeCl_2$	-	-	+	+++	++	++	-
Lead Acetate	++	-	+	++	+	++	+++
PHYTOSTEROLS							
Salkowski's Test	+	-	+	+	+	+	+
Copper Acetate	+	-	++	+++	++	+	+
RESINS							
Acetone-H_2O Test	-	-	-	++	+	+	-
CUMARINS	+	-	+	+	+	-	-
QUINONES	+++	-	+	+	-	++	+++

Table-1 : Phytochemical analysis of different extracts and fractions of *S. glabra*

acetone (Metivier *et al.,* 1980; Ehlenfeldt and Prior, 2001; Nogueira *et al.,* 2008 and Labarbe *et al.,* 1999).

Total Flavonoid Content

The total flavonoid content was reported as Quercetin equivalents with reference to a standard curve (y = 0.1119x - 0.0519, R^2 = 0.9962). The analysis revealed that chloroform: methanolic extract has the highest concentration of flavonoids followed by chloroform extract (Fig. 1c). The fractions isolated from the methanolic extract showed lesser amount of flavonoids as compared to the methanolic extract (Fig. 1d). Earlier studies done on ethanolic extract of *S. japonica* revealed that total flavonoid content was 61.41 ± 1.58 mg catechin

TEST	Hydro-alcoholic A	Hydro-alcoholic B	Chloroform	Methanol	Aqueous	Ethyl Acetate	Butanol	Petrleum Ether	Chloroform : Methanol
ALKALOIDS									
Dragendorff	+++	+++	++	+++	++	+++	++	+++	+++
Mayer's Test	++	+++	+++	+++	+++	++	+++	++	+++
FLAVONOIDS									
$FeCl_2$	-	-	-	-	-	-	-	-	-
Alkaline Reagent	+	+	+	+	++	++	+	++	+
Shinoda	+	+	++	+	+	+	++	+	+
Lead Acetate	+	++	+	++	+	++	++	++	++
CARBOHYDRATES									
Molisch's Test	+++	+++	+++	+++	++	+++	+++	+++	+++
Benedict's	++	+++	++	+++	+++	++	+++	++	+++
Fehling's	+++	++	+++	++	++	++	+	++	++
PHENOLS									
$FeCl_2$	-	-	-	-	-	-	-	-	-
Lead Acetate	+++	++	+++	++	+	++	+	++	++
Iodine	++	++	++	++	++	+	++	+	++
PROTEINS									
Ninhydrin	-	-	-	-	-	-	-	-	-
Xanthoproteic	++	+++	+++	+++	++	+++	++	++	+++
Biuret	++	++	+	++	+++	++	+++	+++	++
SAPONINS									
Foam Test	++	+++	++	+++	+++	++	++	+++	+++
Froth Test	+	+++	+	+++	+++	++	+++	++	+++
GLYCOSIDES									
Keller Killiani	-	-	-	-	-	-	-	-	-
Borntrager	-	-	-	-	-	-	-	-	-
Legal's Test	+++	+++	++	+++	+++	+++	+++	+++	+++
Anthraquinone glycosides	+++	++	++	++	++	++	+++	+++	++
TANNINS									
Gelatin	-	-	-	-	-	-	-	-	-
$FeCl_2$	-	-	-	-	-	-	-	-	-
Lead Acetate	+	++	++	++	+	++	+	++	++
PHYTOSTEROLS									
Salkowski's Test	+	+	+	+	+	+	+	+	+
Copper Acetate	+	+	+	+	+	+	+	+	+
RESINS									
Acetone-H_2O Test	-	-	-	-	-	-	-	-	-
CUMARINS	+	+	+	+	+	+	+	+	+
QUINONES	+++	+++	+++	+++	+++	+++	+++	+++	+++

+++ = Very abundant; ++ =maximally abundant; + = Present; - = absent

Table No. 2: Phytochemical analysis of different extracts

equivalents (Uddin *et al.,* 2016).

***In-vitro* Antioxidant assay**

Earlier, ethanolic extract of *S. glabra* has been reported to exhibit antimicrobial, antioxidant and anti-cancer activity (Semwal, 2009; Lubhan, 2015; Duc *et al*., 2017). ABTS radical scavenging activity revealed that ethyl acetate extract had maximum radical scavenging activity followed by methanolic extract and petroleum ether extract at a concentration range of 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml (Fig 2a, Table 3). Ascorbic assay

Extracts / Fractions	ABTS (IC_{50} µg/ml)
Ascorbic Acid	18.91 ± 1.03
Hydro-alcoholic A (50 methanol:50 water)	794.5 ± 1.43
Hydro-alcoholic B (80 methanol:20 water)	584.9 ± 1.17
Chloroform	119.7 ± 1.03
Methanol	95.19 ± 1.01
Aqueous	135.6 ± 1.07
Ethyl Acetate	**76.31 ± 1.01**
Butanol	660.8 ± 1.28
Petroleum ether	118.8 ± 1.04
Chloroform: Methanol (9:1)	117.2 ± 1.04

Table 3: Inhibitory Concentration (IC50) of different extracts of *S. glabra* as determined by the ABTS, Results represented as mean ± SEM of three different experiments.

Extracts / Fractions	ABTS (IC_{50} µg/ml)
Ascorbic Acid	18.91 ± 1.03
Methanol	**95.19 ± 1.01**
Petroleum ether fraction	127.6 ± 1.106
Chloroform fraction	417.5 ± 1.21
Ethyl acetate fraction	756.7 ± 1.17
Butanol fraction	116.6 ± 1.06
Aqueous fraction	253.8 ± 1.11

Table 4: Inhibitory Concentration (IC50) of different fractions as compared to methanolic extract of *S. glabra* as determined by the ABTS. Results represented as mean ± SEM of three different experiments

was used as positive control in these assays and depicted IC_{50} values of 18.91 ± 1.03 ug/ml in ABTS assay. The plant extracts and fractions possess significant antioxidant potential. The difference in the antioxidant potential is due to the different combination of compounds extracted by different solvents. Methanolic extract has maximum polar compounds whereas petroleum ether extract has non-polar compounds majorly.

Singh *et al* that analyzed ethanolic extract of *S. glabra* possess antioxidant properties and IC_{50} value of 543.16 µg/ml was observed using the DPPH assay (Singh *et al.,* 2015).Sharma *et al* also conducted antioxidant study" on methanolic extract of *S. elegans* by ABTS assay and they found significant radical scavenging activity (IC_{50} 41.66 ± 0.015 µg/ml) of the extract (Sharma *et al.,* 2017)

Anti-cancer Activity

To determine the anticancer potential of various extracts and fractions, MTT assay was performed on A549 (lung carcinomas) cells at concentration range of 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml. After 24 h, cell death analysis was done which revealed that methanolic extract has the best cytotoxic activity (IC_{50} 45.55 ± 1.21 µg/ml) against lung cancer cells with more than 50% cell death (Table 5 and 6). The microscopic examination of the A549 cells also showed the similar results. Water extract and hydro-alcoholic B extract also showed appreciable cancer cell toxicity (IC_{50} 71.61 ± 1.21 and 78.68 ± 1.15 µg/ml) (Fig 3, 4). This data suggests that the solvent used for the extraction of phytochemicals alter the composition of the extracts and hence their bioactivity (Gupta *et al.,* 2012). All the fractions derived from the methanolic extract depicted lower activity as compared to the extract itself (Fig. 3, 4) which suggests that partition of the phytochemicals in various fractions is either reducing the amount of bioactive compounds or the effect shown by methanolic extract is the cumulative effect of various constituents present in the extract (Dai and Mumper, 2010). It has been shown earlier thatn-hexane, ethyl acetate and aqueous fractions of *S. glabra* were cytotoxic against N87, OVCAR-8, MDA-MB-231, HeLa, HepG2 and H358 cell lines *in vitro* and *in vivo* (IC_{50} values 10.27, 12.21 and 18.24 µg/ml) (Duc *et al.,* 2017). Because of the highest anticancer potential shown by the methanolic extract, this extract was used further for the synthesis of the nanoparticles.

Anti-Microbial Assay

The anti-microbial activity of different extracts and fractions of *S. glabra* was determined by colony forming unit assay (CFU) at concentration range of 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml using *B. subtilis and P. aeruginosa* bacteria. The growth curve of bacteria was also analyzed and the effect of extracts and fractions on the growth of bacteria was determined. MIC is the least concentration of extract inhibiting the growth of the test

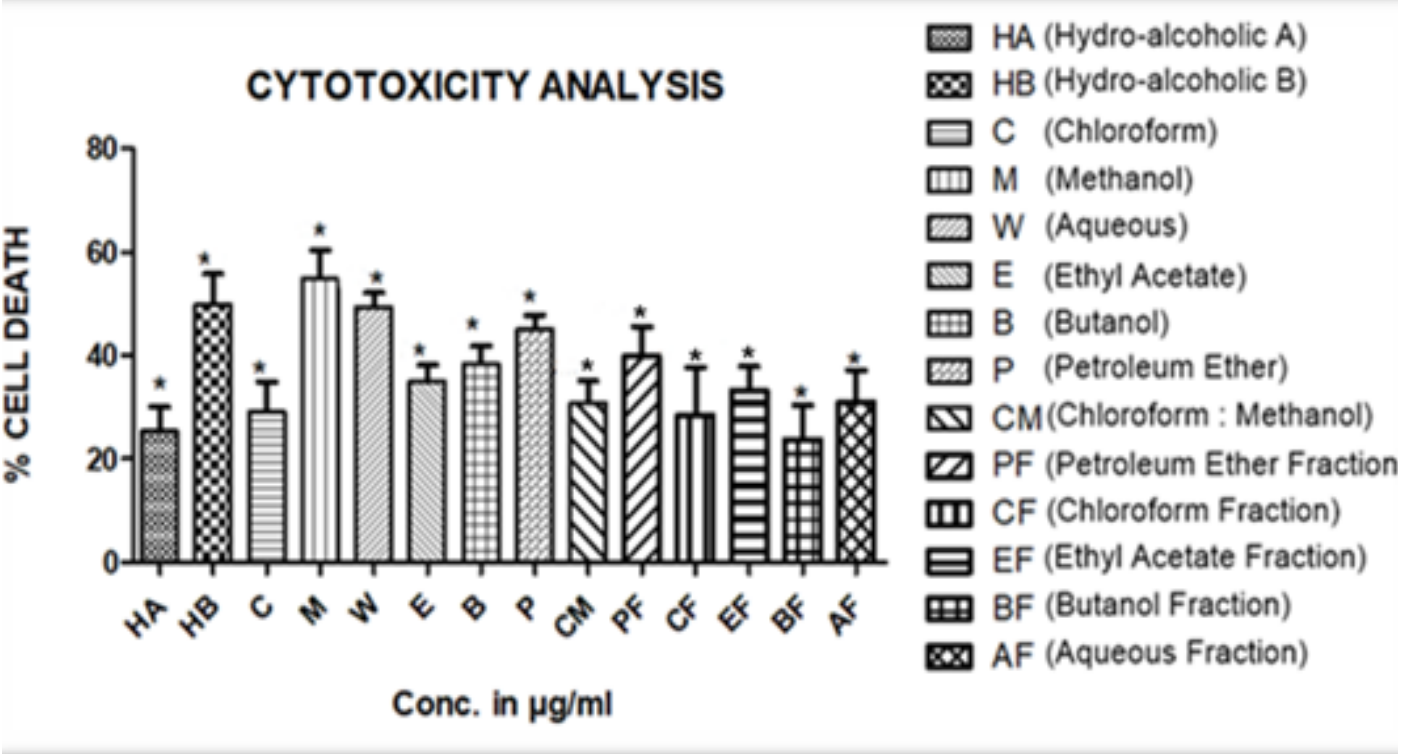


Fig 3: In-vitro evaluation of the cytotoxicity of different extracts and fractions of S. glabra against A549 cell lines. Results represented as mean ± SEM of three different cultures. Data is statistically significant with *p < 0.05 when compared with control.

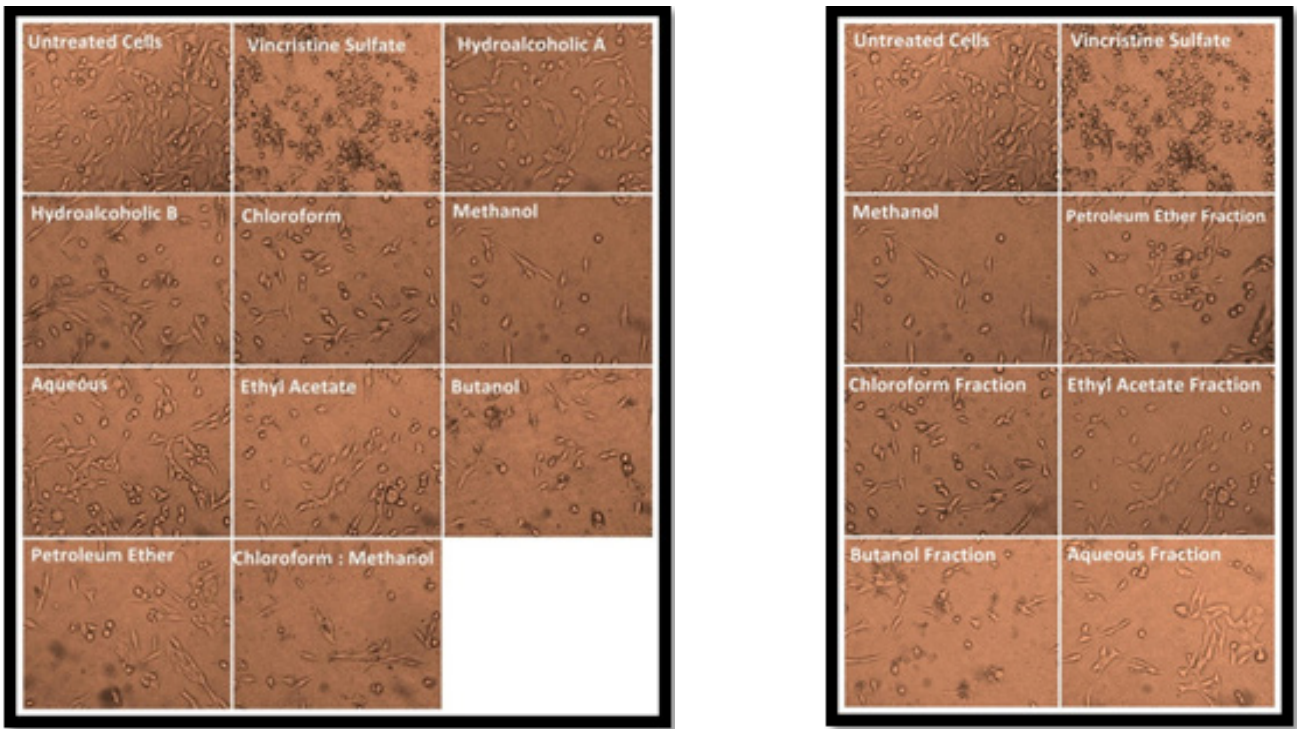


Fig 4: Representative microscopic images at 10X, depicting morphology of untreated cells, cells treated with vincristine sulfate (4 µg/ml) and cancer cells treated with different extracts and fractions of S. glabra (200 µg/ml) after 24 h treatment.

organisms during 24 h. These values were further used for analysis of colony forming unit and growth curve analysis. The extracts/ fractions were found active against *B. subtilis* with MIC range of 6.25 – 50 mg/ml and against *P. aeruginosa* with MIC range of 12.5 – 50 mg/ml (Table 7, 8). Previously, ethanol extract of *S. glabra* was found to be most active extract out of *n*-hexane, acetone and ethanol extracts. The maximum inhibition zone was against the bacterial strains *Streptococcus mutans* and *Staphylococcus epidermidis* with MIC of 50 µg/ml. It was also active against the hospital strains of *Staphylococcus aureus* (Semwal *et al.,* 2009).

Colony Forming Unit (CFU)

Extract / Fraction	IC_{50} (µg/ml)
Hydro-alcoholic A (50 methanol:50 water) – HA	545 ± 1.2
Hydro-alcoholic B (80 methanol:20 water) - HB	71.61 ± 1.21
Chloroform – C	307.4 ± 1.14
Methanol – M	**45.55 ± 1.21**
Aqueous - AQ	78.68 ± 1.15
Ethyl Acetate – E	515.7 ± 1.34
Butanol – B	320.2 ± 1.25
Petroleum ether - P	163.4 ± 1.24
Chloroform: Methanol (9:1) - CM	563.4 ± 2.1

Table 5: Inhibitory Concentration (IC50) of different extracts of S. glabra as determined by the (anti-cancer) MTT assay, Results represented as mean ± SEM of three different experiments.

Extract / Fraction	IC_{50} (µg/ml)
Methanol – M	**45.55 ± 1.21**
Petroleum ether fraction – PF	464.9 ± 1.36
Chloroform fraction – CF	294 ± 1.38
Ethyl acetate fraction – EF	288.6 ± 1.40
Butanol fraction – BF	182 ± 1.21
Aqueous fraction – AF	166.8 ± 1.17

Table 6: Inhibitory Concentration (IC50) of different fractions as compared to methanolic extract of *S. glabra* as determined by the (anti-cancer) MTT assay, Results represented as mean ± SEM of three different experiments.

Extract / Fraction	***B. subtilis*** **(mg/ml)**	***P. aeruginosa*** **(mg/ml)**
Hydro-alcoholic A (50 methanol:50 water) – HA	6.25	12.5
Hydro-alcoholic B (80 methanol:20 water) – HB	25	12.5
Chloroform – C	25	25
Methanol – M	25	25
Aqueous - AQ	50	50
Ethyl Acetate – E	25	25
Butanol – B	6.25	12.5
Petroleum ether – P	50	25
Chloroform: Methanol (9:1) – CM	12.5	25

Table 7: MIC values of different extracts of *S. glabra* against B. subtilis and *P. aeruginosa.*

Extract / Fraction	*B. subtilis* (mg/ml)	*P. aeruginosa* (mg/ml)
Methanol – M	25	25
Petroleum ether fraction – PF	25	25
Chloroform fraction – CF	12.5	12.5
Ethyl acetate fraction – EF	25	25
Butanol fraction – BF	25	25
Aqueous fraction – AF	25	25

Table 8: MIC values of different fractions as compared to methanolic extract of *S. glabra* against B. subtilis and *P. aeruginosa.*

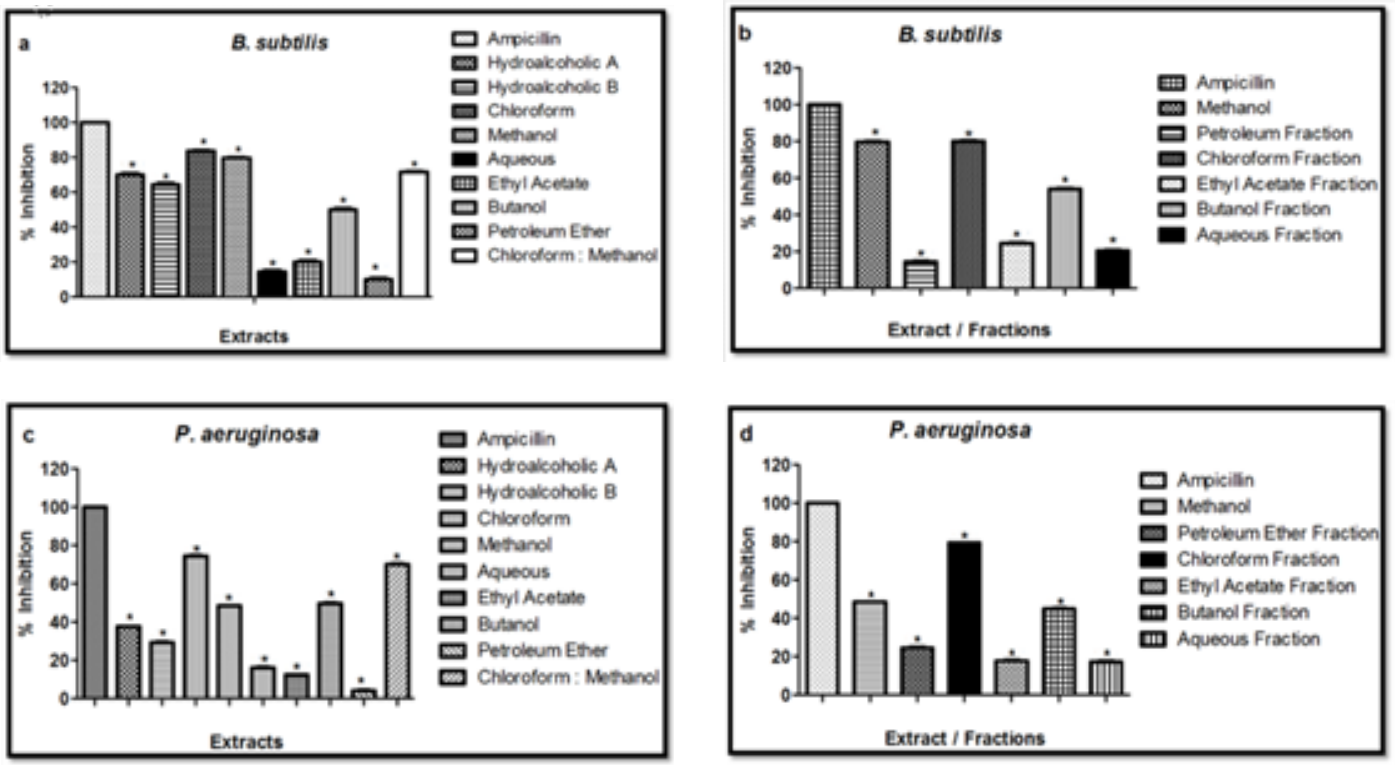


Fig 5: Percentage inhibition in the growth of *B. subtilis* (a) and P. aeruginosa (c) by different extracts of *S. glabra.* Percentage inhibition in the growth of B. subtilis (c) and P. aeruginosa (d) by different fractions as compared to methanolic extract of *S. glabra.* Results represented as mean ± SEM of three different experiments. Data is statistically significant with *p < 0.05 when compared with control.

The colonies of *B. subtilis* were inhibited maximally by chloroform extract and fraction followed by methanolic extract and chloroform: methanolic extract. Chloroform fraction inhibited the colonies of *P. aeruginosa* maximally with a relative inhibition by chloroform and chloroform: methanolic extract followed by methanolic extract (Fig 5, 6). The antimicrobial activity of chloroform extract/ fraction can be attributed to the presence of certain non-polar compounds exhibiting this activity. The number of bacterial colonies was minimum in both the cases upon treatment with chloroform extract/ fraction (Chandna *et al.,* 2019). In case of B. subtilis chloroform has the maximum percentage, inhibition followed by chloroform fraction and methanol extract. In case of P. aeruginosa chloroform extract has the maximum percentage, inhibition followed by chloroform fraction and chloroform: methanols which have comparable percentage inhibition. Ethanolic extract of *S. glabra* was tested for its antimicrobial activity and was found to inhibit the growth of *Staphylococcus aureus, Escherichia*

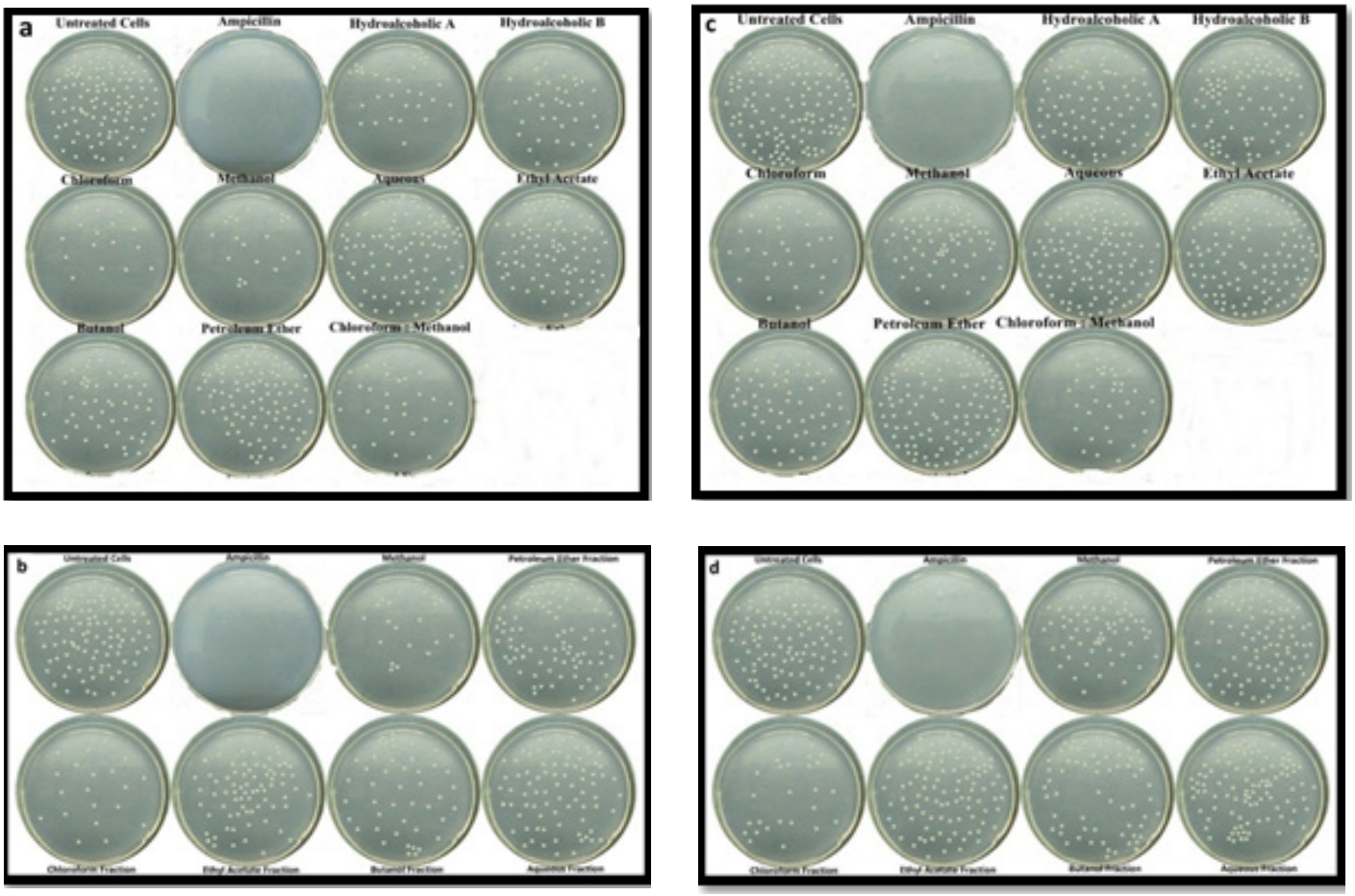


Fig 6: Inhibition of colonies of *B. subtilis* (a and b) and P. aeruginosa (c and d)when treated with different extracts of *S. glabra* (a, c)and different fractions as compared to methanolic extract of *S. glabra* (b, d).

coli, Streptococcus mutans, Staphylococcus epidermidis and *Klebsiella pneumonia* bacteria as exhibited by significant inhibitory zone diameter (Semwal *et al.,* 2009)

CONCLUSION

The extracts and fractions of *S. glabra* showed significant antioxidant activity and hence can be used as source of natural antioxidants for curing diseases caused by oxidative stress. The study also determined promising anticancer potential of plant which could be used as a source of novel drug to fight cancer. Also this plant has potential antimicrobial activities against pathogenic bacteria. Much interest is given these days towards naturally-derived phytocomponents as they are considered to have less side effects compared to current treatments such as chemotherapy. Also, there is a growing demand for alternative treatments with naturally-derived anticancer agents with plants being the desired source. Medicinal plants like *S. glabra* can be of significant use in this context. However, further investigations need to be carried out to isolate and characterize the specific bioactive compounds responsible for such activities. The plant extract and the isolated compounds can be utilized to prepare nano-formulations effective against various ailments. Moreover, *in vivo* studies are also necessary to confirm the anticancer potential of this plant in more detail.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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