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Full Length Research Article

Identification, Quantification, Antimicrobial and Antioxidant Activities of Ethanolic Stem Extract of *Sida acuta* Burm F.

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Abstract

Herbal plants are affluent resource of bioactive compounds with various pharmaceutical applications. Qualitative phytochemical analysis, Quantitative analysis, antimicrobial activity and antioxidant activity were analyzed in ethanolic stem extract of *Sida acuta* Burm F. Qualitative phytochemical screening was done to identify the presence and absence of phytochemicals. Carbohydrates, protein and amino acid were estimated in the range of 51.53 µg/mg, 38.66 µg/mg and 30.60 µg/mg respectively. Likewise total phenol total tannin, total alkaloid and total flavonoid was identified in the order of 1.83 ± 0.02, 1.66 ± 0.064, 1.55 ± 0.040 and 1.53 ± 0.07 (100 µl in 10 mg/ml). Maximum inhibition was found against growth of bacteria like *Bacillus subtilis, Staphylococcus aureus,* and *E. coli*. As well fungi like antifungal activity of ethanolic stem extract of *S. acuta* were revealed better zone of inhibition against *Aspergillus niger* and *Rhizopus stolonifer* species. Concentration-reliant reducing capacity was observed at 100 µl in 10 mg/ml (1.80 ± 0.02). From the results of present study, it can be concluded that ethanolic stem extract of *Sida acuta* Burm F. has the ability to act against various microbial infections as well as free radicals responsible for the generation of numerous diseases.

***Key words*:** Qualitative phytochemical analysis, Quantitative analysis, Antimicrobial activity, Antioxidant activity, *Sida acuta* Burm F.

Contagious disorders are main trouble in the globe. Annually 57 million people die owing to these diseases [1]. Nowadays multi-drug resistant (MDR) microbes are emerging and also restricting the activity of antibiotics [2]. So, there is a need for effective medications with lower or without side effects. As a result of metabolism, free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced. They are toxic and also responsible for the cause of chronic illnesses because of damaging macromolecules. These free radicals are scavenged through the activity of antioxidants. Generally, antioxidants are classified as synthetic and natural antioxidants. Exogenous or natural antioxidants from diet can be suggested because of toxic effect of synthetic antioxidants [3]. So, it is replaced by means of natural antioxidants [4]. Many human ailments have been treated with the use of medicinal herbs. Phytochemical compounds present in these plants that can create changes in human health for example alkaloids, flavonoids, terpenoids, tannins and saponins. Those compounds which can be helpful to develop pioneer pharmaceutical medications for suppression of bacterial and fungal organisms as well free radical scavenging [5]. The main objective of the present work was to study qualitative, quantitative phytochemical analysis, antimicrobial in addition with antioxidant activities of ethanolic extract of *Sida acuta* Burm F. stem.

MATERIALS AND METHODS

Fresh stem of *Sida acuta* Burm F. was collected from in and around region of Thanjavur, Tamil Nadu and its shade dried for two weeks to prepare fine powder. This powder was soaked in absolute ethanol for five days. The mixture was filtered then used for qualitative, quantitative phytochemical analysis, antimicrobial as well antioxidant activity assays.

Qualitative phytochemical analysis

Preliminary phytochemical screening was done using the method given by Auwal *et al*. [6].

Test for carbohydrate

In stem extracts (2 ml), molisch reagent (1 ml) as well small amount of H_2SO_4 (concentrated sulphuric acid) were

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added. After addition, identification of red shade indicates the existence of carbohydrate.

Test for protein

Few ml of extracts was mixed with NaOH (10%, sodium hydroxide) and two drops of CuSO4 (0.1%, copper sulphate). The observation of pink colour indicates the occurrence of protein.

Test for tannins

In stem extracts (1 ml), 5% $Fecl_3$ (2 ml, ferric chloride) was added. It leads to the formation of light blue or dark green colour confirms tannin existence.

Test for saponins

Distilled water (2 ml) was combined with stem extracts (2 ml) then shake it. After 15 minutes, foam like appearance reveals incidence of saponins.

Test for alkaloids

With stem extracts (2 ml), 2 ml of concentrated hydrochloric acid (Hcl) was mixed. In this mix, little droplet of Mayer's reagent was mingled. White or green precipitate reveals alkaloids existence.

Test for flavonoids

In stem extracts (2 ml), 1 ml of 2N sodium hydroxide (NaoH) was added. It leads to the development of yellow colour indicates occurrence of flavonoids.

Test for phenol

In stem extracts (1 ml), small amount of phenol Ciocalteau reagent subsequently sodium carbonate (15%) was mixed. After addition, green or blue colour formation reveals the incidence of phenols.

Test for glycosides

Addition of 3ml chloroform and ammonia solution (10%) with stem extracts (2 ml) develop pink colour. It demonstrates the existence of glycosides.

Test for terpenoids

With stem extracts (0.5 ml), chloroform (2 ml) then concentrated H_2SO_4 was closely combined into sides of the test tubes. At the interface, brown color development indicates presence of terpenoids.

Test for quinones

Concentrated sulphuric acid (1 ml) was blended with 1ml of stem extracts. The formation red colour development shows the occurrence of quinones.

Test for coumarins

1ml of 10% of sodium hydroxide was mingled with stem extract (1 ml). The formation of yellow colour indicates coumarin existence.

Test for steroids

Equivalent quantity of chloroform was added with 1ml of stem extracts. Then small amount of H_2SO_4 (concentrated) was mixed. Existence of brown colour ring indicates incidence of steroids.

Test for phlobatannins

With stem extracts (1 ml), droplet of hydrochloric acid (2%) was added. Red color precipitate suggests the existence of phlobatannins.

Quantitative phytochemical analysis

Estimation of total carbohydrate

Anthrone method was used to measure total carbohydrate [7] in ethanolic extract of *S. acuta* Burm F. Aqueous and hot water extracts (10mg/ml) as well as standard (10mg/10ml) were used to identify the level of carbohydrate in it. Preparation of anthrone reagent: In ice cold concentrated sulphuric acid (H_2SO_4), anthrone (0.2g) was dissipated.

Different concentrations of extract and Standard (20, 40, 60, 80 and 100 µl) were taken in test tubes and dissolved using distilled water (up to 1ml). In this mix, anthrone reagent (4ml) was added, warmed then cool down. After cooling, bluish green colour was appeared. Optical density (OD) values were deliberated at 630nm. Dextrose was used as standard. Blank also prepared using above steps instead of extract. Calculation was done using given formula:

Amount of Glucose = Test absorbance / Standard absorbance × Standard concentration

Estimation of total amino acids

Total amino acid quantification was done using the method given by Trease and Evans [8]. Ethanol, water and hot water extract (10mg/ml) as well as Standard (10mg/10ml) was used to estimate the amount of amino acids in it. Preparation of Ninhydrin Reagent: 500 ml of citrate buffer (0.2M) with stannous chloride (0.8 g) and 500 ml of 2 methoxy ethanol with ninhydrin (20 g). Diluent solution was made by mixing equal volume of water and n-propanol.

Various concentrations of extract and Standard (20, 40, 60, 80 and 100 µl) were taken and it was made with distilled water upto 1ml. In these test tubes, 1 ml of ninhydrin reagent was mixed. It was incubated in water bath. After 20 minutes, diluent solution (5 ml) was appended to all tubes. Absorbance was read at 570nm. Leucine was utilized as standard.

Estimation of total protein

Protein content was measured using the method suggested by Bradford [9]. Bradford Reagent: Coomassie Brilliant Blue G-250 (100 mg) was dispersed in 95% ethanol (50 ml). In this mix, Phosphoric acid (100 ml, 85%) was added. Various concentrations of extracts and Standard (20-100 µl) were taken and it was made up to the mark of 1 ml. Then Bradford reagent (5 ml) was combined with the above mixture. Then optical density values were estimated at nm. Calculation was done using below formula:

Amount of Glucose = Test OD / Standard OD × Standard concentration

Estimation of total alkaloid

BCG solution: Bromocresol green (69.8 mg) with NaOH (2N, 3 ml) and distilled water (5 ml) was heated to dissipate this mix then it was made up to 1000 ml using distilled water. Phosphate buffer (pH 4.7): pH of sodium phosphate (2M) was adjusted to 4.7 through the combination of citric acid (0.2 M). Caffeine standard: atropine (0.5 mg) was mixed in 5 ml distilled water. Total alkaloid was estimated with the help of method reported by Ajanal *et al*. [10]. 2N HCl was used to dissolve the extract afterwards sieved. Various concentrations of extract and standard was taken (20, 40, 60, 80, 100 µl) in reparatory funnels. In this mix, BCG (Bromocresol green, 5 ml) as well phosphate buffer (5 ml) was combined. It was agitated with the addition of chloroform (1, 2, 3, and 4 ml) then collected in volumetric flask (10 ml). Finally, it was adjusted with chloroform. Absorbance was calculated at 470 nm. Results were expressed as µg caffeine equivalents (CE)/mg.

Estimation of total flavonoid

Aluminium chloride method was exploited to estimate flavonoid content [11]. Quercetin was used as standard. The extract (10mg/ml) and quercetin (10mg/10ml) were prepared. Standard as well as extracts were taken in the concentrations of 20-100 µl. Then distilled water (4 ml), 10% sodium nitrite (0.3 ml) in addition with 10% aluminium chloride (0.3 ml) was added and incubated for 6 minutes. Subsequently sodium hydroxide (2 ml, 1%) was mixed. OD values were caliberated at 510 nm and they were given as µg quercetin equivalents (QE)/ mg.

Estimation of total tannin

This method was done using the method given by Kavitha Chandran and Indira [12]. Folin ciocalteau technique was used to determine tannin content in ethanol, water and hot water stem extracts. Standard (Tannic acid) as well as extracts were taken in the concentrations of 20-100 µl respectively. Then extract was combined with distilled water (7.5 ml), Folin Ciocalteu phenol reagent (0.5 ml), sodium carbonate solution (35%, 1 ml) in addition with distilled water was used for dilution up to 10 ml. Then it was incubated (37°C, 30 min). Optical density was estimated at 700 nm. Total tannin was represented in terms of as µg TE/mg.

Estimation of total phenol

Total phenol was assayed according to method suggested by Dewanto [13]. Standard (Gallic acid, 10mg/10ml) and ethanol stem extracts (10mg/ml) were used to determine the level of phenols. Different test tubes with extracts in the range of 20-100 µl were taken. Extract was mixed with distilled water (0.5 ml) as well Folin-Ciocalteu reagent (0.125 ml). After 6-minute incubation with shaking, Na_2CO_3 (7%, 1.25 ml) was added. Then this mix was made up to 3 ml with the combination of distilled water and incubated in shady place. Optical density was read at 760 nm. Phenol content was expressed as µg GE/mg.

Antimicrobial activity

Agar well diffusion method [14] was applied to analyze antimicrobial activity of stem extract at the volume of 40 µl and 80 µl for each bacterial as well fungal microorganism. Bacteria used for antibacterial activity were *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas putida*. Antifungal capacity of the extract was found against *Aspergillus niger*, *Rhizopus stolonifer* and *Trichoderma viride*.

Antibacterial activity

Test microorganisms

Four types of bacteria were utilized to identify antibacterial activity of ethanolic stem extract of *Sida acuta* Burm F. They are gram positive (*Staphylococcus aureus, Bacillus subtilis*) and gram-negative bacteria (*Pseudomonas putida, Escherichia coli*).

Antibacterial activity assay

10 ml of nutrient broth was taken in test tubes. In these tubes, bacterial inoculums were added. Then it was set aside for 24 hrs at 37°C.

Nutrient agar (25ml) was transferred into presterilized Petri dishes and allows solidifying at room temperature for 1hr. After solidification, bacterial cultures were spread into nutrient agar separately. It was then punched with a needle to prepare wells. Three wells were created. In that one well was kept as control and other wells were then filled with different concentrations (40 and 80µl) of the plant extracts. Petri dishes with bacterial cultures was kept at incubator (37°C, 24 hrs. The inhibition zone diameter (IZD) was detected by deducting the diameter of well.

Antifungal activity

Test microorganisms

Different fungal strains were taken to analyze antifungal activity of ethanolic stem extract of *Sida acuta* Burm F. The fungal organisms employed were *Aspergillus niger*, *Rhizopus stolonifer*, *Trichoderma viride*.

Antifungal activity assay

Separate PDA (Potato Dextrose Agar) plates were used to grow fungi at 28 °C then it was sustained with intermittent sub-culturing (4 °C). The PDA medium was pelt down into the sterilized Petri plates. Subsequently medium was allowed to become solid. Individual fungal cultures were spread on the medium using cleaned buds. Cork borer was used to create 6 mm wells. Three wells were made. In that wells, one well was kept as control and other wells were filled with different concentrations (40 and 80µl from 5 mg/ml) of the plant extracts. The plates were kept at 27°C for two days. After that clear zone was monitored surrounding the well. Clear zone of inhibition specifies the effect of extracts against specific fungal microorganisms used. The zone of inhibition was recorded.

Antioxidant activity

Reducing power assay

Reducing power was done with the help of Jeyaprakash *et al*. [15] method. This assay was utilized to analyse the capacity of antioxidant present in extract through the formation of TCA, $FeCl_3$ (ferric chloride) and potassium ferricyanide. Extracts with different concentrations like 20, 40, 60, 80 and 100 µl were taken and it was combined with potassium ferricyanide and phosphate buffer (pH 6.6). This mixture was kept for 20 minutes at 50°C. 10% trichloroacetic acid (TCA) was mixed, centrifuged (10 min, 3000 rpm). Finally supernatant was mingled with 0.1% ferric chloride. Absorbance was noted at 700 nm.

Total antioxidant activity

Total antioxidant activity was performed using the method of Prieto *et al*. [16] with some changes. Various concentrations of extracts (20-100 µl) as well standard (ascorbic acid) were taken in test tubes. This mixture was mingled with reagent solution (1 ml) containing 0.6 M sulphuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate. Then these tubes were incubated for 90 minutes at 95°C and cooled. OD values were calculated at 695 nm.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

Aqueous and hot water extracts of *Sida acuta* stem were chemically tested for the presence of different phytochemical constituents such as carbohydrates, protein, alkaloids, flavonoids, phenols, glycosides, cardiac glycosides, steroids, terpenoids, saponins, tannins, quinines, anthraquinones, coumarins and phlobatannins (Fig 1-3). Water and hot water stem extracts were showed the presence and absence of some phytoconstituents (Table 1).

Fig 1 Identification of alkaloids, flavonoids, Quinones in water and hot water extracts of *Sida acuta* Burm F.

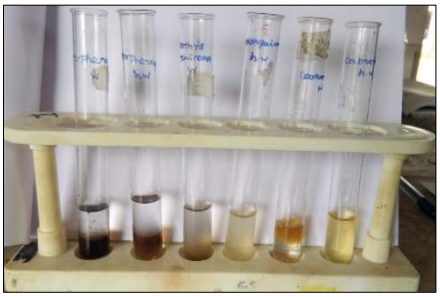

Fig 2 Identification of terpenoids, anthraquinones, coumarins in water and hot water extracts of *Sida acuta* Burm F.

Fig 3 Identification of tannin, saponin in water and hot water extracts of *Sida acuta* Burm F.

Table 1 Qualitative analysis of primary and secondary metabolites in various extracts of *S. acuta* Burm F. stem.

Phytochemical compounds	Extracts	
	Aqueous extract	Hot water extract
Carbohydrates	+	+
Protein	+	+
Alkaloids	+	+
Flavonoids	++	+
Steroids	+	-
Terpenoids	+	-
Quinones	+	-
Anthraquinones	+	-
Glycosides	+	-
Saponins	+	-
Tannins	+	+
Phenols	+	+
Coumarins	+	+
Phlobatannins	+	-

+ - Present, ++ - Moderately Present, - - Absent

Quantitative analysis

Primary and secondary metabolites

Estimation of Primary metabolites present in ethanolic stem extract of *Sida acuta* Burm F. was done and the results are represented in (Fig 4). Quantitative estimation of carbohydrates, protein and amino acid was in the range of 51.53 µg/mg, 38.66 µg/mg and 30.60 µg/mg respectively.

Secondary metabolites

Secondary metabolites like alkaloid, phenol, flavonoid and tannin were estimated in ethanol extract of *Sida acuta* Burm F. stem. Total phenol content was found more (1.83 ± 0.02 at 100 µl in 10mg/ml) at higher concentration of extract. Likewise total tannin, total alkaloid and total flavonoid was identified in the order of 1.66 ± 0.064, 1.55 ± 0.040 and 1.53 ± 0.07 (100 µl in 10mg/ml). When concentration of extract increased, the level of secondary metabolites also increased that is shown in (Fig 5).

Antimicrobial activity

Antibacterial activity

Ethanolic stem extract of *S. acuta* Burm F. was showed significant growth inhibition of bacteria like *Staphylococcus aureus*, *Bacillus subtilis* and *E. coli* which is exhibited in (Fig 6). These results are supported by the work on ethanolic leaf extract of *S. acuta* Burm F. [17]. Maximum zone of inhibition was observed with gram positive bacteria because of sensitivity on the extract and this is also previously reported by Akilandeshwari *et al.* [18].

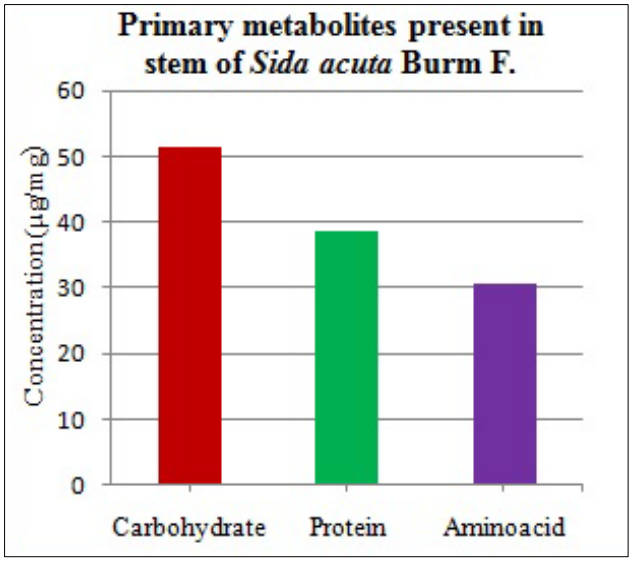

Fig 4 Quantitative analysis of primary metabolites

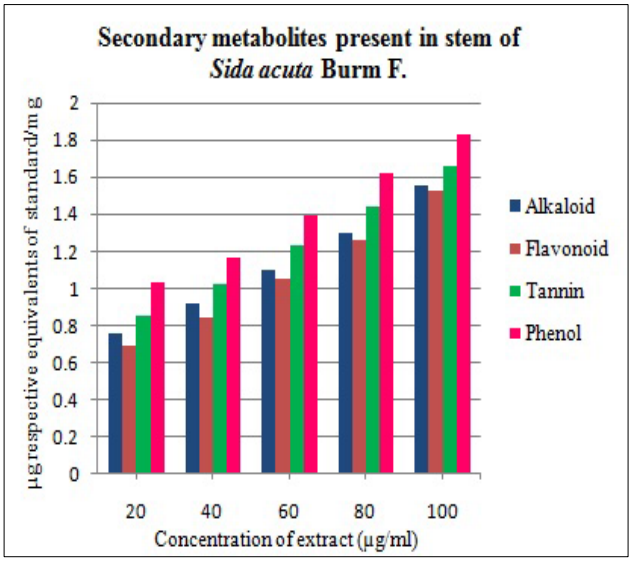

Fig 5 Availability of total alkaloid, flavonoid, tannin and phenol in stem extract of *S. acuta* Burm F

Antifungal activity

Antifungal activity of ethanol extract of *Sida acuta* Burm F. were revealed better zone of inhibition against *Aspergillus niger* which is represented in (Fig 7) and this result is supported by the work on ethanolic leaf extract of *Sida acuta* Burm F. [17]. Similarly better inhibition was found against *Rhizopus stolonifer* species.

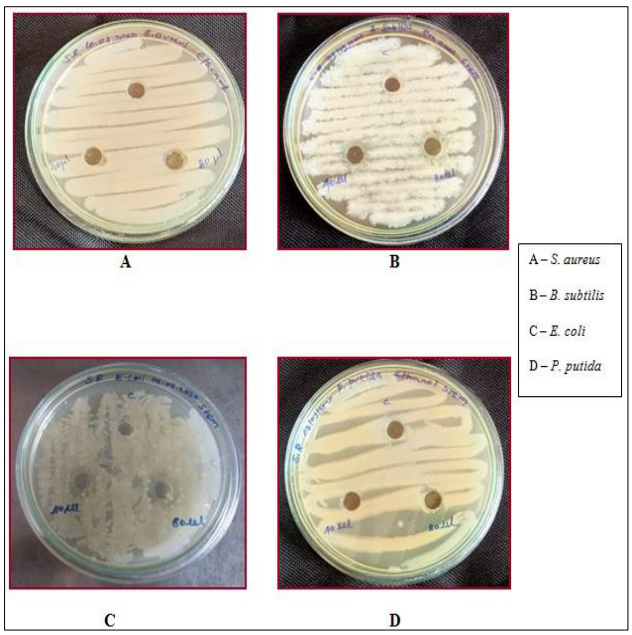

Fig 6 Effect of ethanolic stem extract of *S. acuta* Burm F. against different bacteria

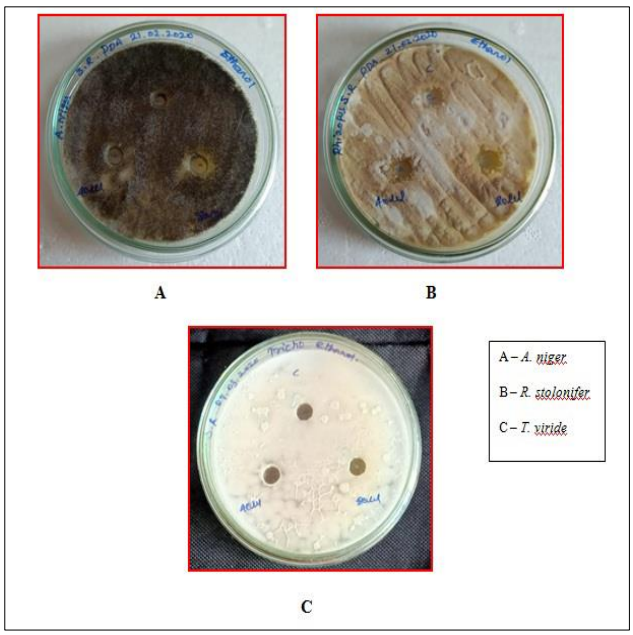

Fig 7 Antifungal activity of ethanolic stem extract of *S. acuta* Burm F.

Antioxidant activity

From the results of reducing power assay, stem extract revealed concentration-reliant reducing capacity at 10mg/ml (1.80 ± 0.02) and it is shown in (Fig 8). It exhibits extract can act as electron donors and also decrease the level of lipid peroxidation. Phosphomolybdenum method was exploited to identify the antioxidant activity of this extract. In case of acidic state, molybdenum VI was reduced into green colour phosphomolybdenum (V) complex that is directly proportional to the capacity of antioxidant present in extract. Antioxidant property of extract was observed at the range of 1.78 ± 0.038 in ethanol extract. Concentration dependent antioxidant activity was depicted in (Fig 9).

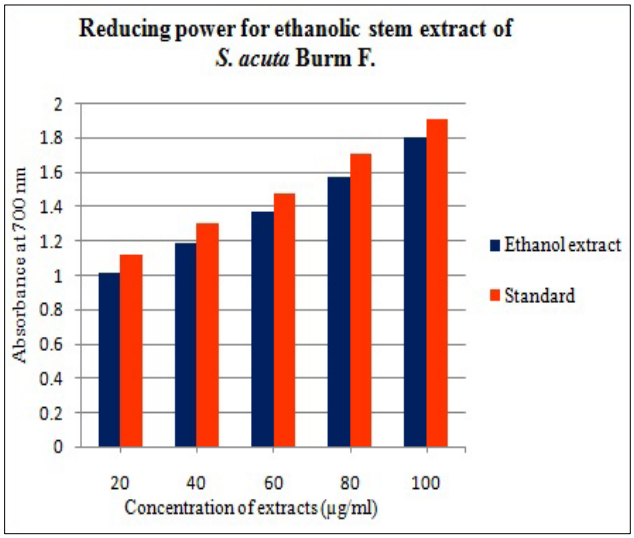

Fig 8 Fe^{3+} reducing ability of *S. acuta* Burm F. stem

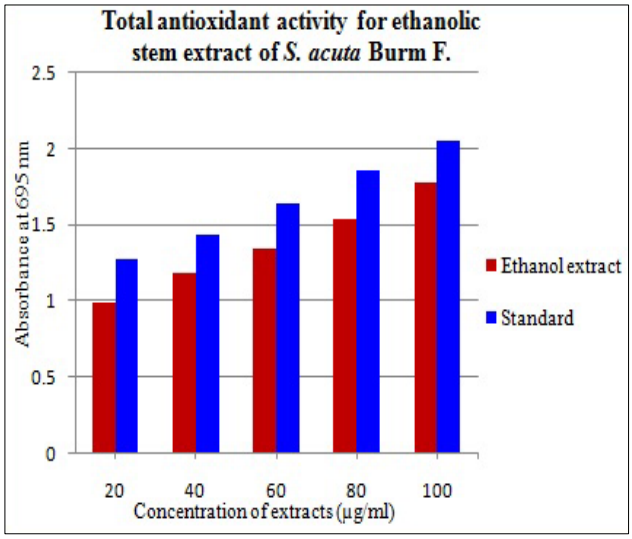

Fig 9 Antioxidant capacity of ethanolic stem extract of *Sida acuta* Burm F.

Antioxidants are substances with less MW (molecular weight) have the capacity to interfere and cease series of free radical formation events for hindering cell destruction [19]. Naturally antioxidants have free radical quenching, hydrogen and electron endowment along with metal chelating properties [20].

Electron donating capacity of antioxidant available in extract has been identified using reducing power method [21]. As a consequence of this assay, ferric cyanide (Fe^{3+}) can be downgraded into ferrous cyanide (Fe^{2+}) through altering the colour of solution (green to blue) [22].

CONCLUSION

The present study indicates that the stem extract of *Sida acuta* Burm F. have phytochemicals like alkaloid, flavonoid, terpenoids, tannins, steroids, saponin, phenolic compound, quinone, anthraquinone, glycosides, carbohydrate and protein. Due to the presence of more alkaloids, flavonoids and phenolic compounds, stem extract has antimicrobial as well antioxidant activity. So, stem of this plant can be used to prepare new-fangled drugs in pharmaceutical industries. Further examinations will be needed to identify which compound of stem is responsible for antimicrobial and antioxidant activities.

Author contributions: Author contributed to the paper

Conflict of interest: Nil

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