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Phytoconstituents from fruit parts of *Tiliacora acuminata* (Lam.)Hook. f. &Thoms and *Stephania wightii* (Arn.) Dunn (Menispermaceae) with *invitro* antioxidant activity

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Abstract

The present study was carried out to investigate the phytochemical and *invitro* antioxidant activity of fruit parts of *Tiliacora acuminata* (Lam.) Hook. f. & Thoms and *Stephania wightii* (Arn.) Dunn (Menispermaceae) are important medicinal plants. Qualitative phytochemical analysis of the petroleum ether, ethyl acetate, chloroform, ethanol and aqueous extract of fruit part of both plants revealed the presence of alkaloids, phenols, tannins, glycosides. In fruit part of *S. wightii* resins and steroids are present and in *T. acuminata* cardiac glycosides are present. Antioxidant activity of petroleum ether, ethyl acetate, acetone and ethanol extracts of the fruit part of *T. acuminata* and *S. wightii* have been tested using various antioxidant model systems viz, DPPH, ABTS, Ferrous iron chelating activity and reducing ability. This study indicates significant free radical scavenging potential of *T. acuminata* and *S. wightii* fruit which can be exploited for the treatment of various free radical mediated ailments.

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1. Introduction

Herbal plants are the sources of crude drugs that are used to treat various diseased conditions. A number of cultures have distinct uses of plants for the treatment of various diseases. This traditional knowledge has been vocally passed on through the generation to generations; therefore people still in practice traditional remedies (Waqas *et al.*, 2016). Recent investigations have shown that the positive antioxidant properties of medicinal plants could be interrelated with bioactive phytoconstituents such as alkaloids, flavonoids, bioflavonoids, glycosides, coumarins, terpenoids,

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saponins, tannins and phenols (Nishanthini *et al.,* 2016).

The genus *Tinospora* and *Stephania* belongs to the family Menispermaceae, *Tinospora* genus has been reported to contain immunomodulatory constituents (Veena *et al.,*2002), anti-diabetic anti-dyslipidemic potential and rich source of alkaloid and terpenes. In modern medicine it is called as the magical rejuvenating herb. The stem is highly nutritive, digestive and contains rich amount of berberine, palmatine, tembetarine, magnoflorine, tinosporin, tinocordifolin. The stem owing to its higher alkaloid content is approved for many medicinal usage (Kuhu *et al.,* 2015).The genus *Stephania* have recognized medicinal values and traditionally have been used for the treatment of asthma, tuberculosis, dysentery, hyperglycemia, cancer, fever, intestinal complaints, sleep disturbances and inflammation (Deepak *et al.,* 2010). The present study was attempt to analyse the phytochemical and *invitro* antioxidant activity of fruit parts of *T. acuminata* and *S. wightii* from Menispermaceae family.

1. Materials and methods

1.1. Collections of plant material

Fruit of *Tiliacora acuminata* (Lam.) Hook. f. &Thoms and *Stephania wightii* (Arn.) Dunn were collected from Western Ghats region of Nilambur, Malappuram district, Kerala, India. They were identified and authenticated by Taxonomist, Dr. Binu Thomas PG & Research Department of Botany, St. Joseph's College (Autonomous), Devagiri, Kozhikode, Kerala, India and voucher specimen has been deposited in Kongunadu Arts and Science College, Coimbatore.

. **Description of the selected plants**

Botanical Name:	***Tiliacoraa cuminata*** (Lam.)Hook. f. &Thoms	***Stephania wightii*** (Arn.) Dunn
Description:	Climbing shrubs; stems striate, sparsely puberulous or glabrous. Leaves alternate, ovate or lanceolate, truncate, cordate, rarely acute at base, acuminate at apex, 8-14 x 3.5- 8 cm, chartaceous, glabrous, 3-5 nerved at base; petioles 1.5-3 cm long, sulcate, glabrous. Inflorescences axillary, panicled, 3.5-10 cm long, pubescent. Male flowers 2-7 at apex of inflorescence, yellow; sepals 6 in 2 rows; inner ones	Creeping herbs. Leaves 8 cm across, orbicular, glaucous, margins wavy or coarsely dentate; petiole 8 cm long, slender. Peduncle to 2 cm long, erect; pedicel 3 mm long; male flowers densely packed in heads,; female flowers 3 mm across, in pedunculate cymes; sepals 5, ovate acute glabrous; petals ovate, greenish yellow.

	broadly elliptic, glabrous; petals 6, obovate, glabrous; stamens 6, cylindric. Female flower solitary; sepals and petals as in male ones; carpels 8-12, glabrous, on stalked puberulous gynophore. Drupes on branched carpophores, oblong to obovoid, 10-15 x 6-7 mm, glabrous, red when ripe; endocarp reticulate	Drupe 8 x 5 mm, oblique, stalked, with 4 rows of tubercles along the margins
Habit:	Climber (**Fig. 1-2**)	Climber (**Fig. 3-4**)
Flowering:	April-December.	April-June
Habitat:	Moist deciduous forests and also sacred groves in the plains	Evergreen and semi-evergreen forests, especially in rock crevices
Distribution:	India, Sri Lanka and South East Asia	India and Malesia

Fig 1. Habit of *Tiliacoraa cuminata*

Fig 2. Fruit part of *Tiliacora acuminata*

Fig 3. Habit of *Stephania wightii*

Fig 4. Fruit part of *Stephania wightii*

1.2. Preparation of plant extract

The fresh fruit parts of *T. acuminata* and *S. wightii* were washed with tap water and shade dried for a week and powdered coarsely. Then they were finely powdered mechanically using pulverizer and passed through 40mesh sieve and stored in airtight containers. About 25g of powdered fruit parts were extracted by using shaker apparatus with petroleum ether, ethyl acetate, acetone and ethanol. The extract was dried under reduced pressure at low temperature (40-50°C). The last traces of the solvent were removed under vacuum drier andthe solid mass obtained was stored at 4°C until further use.

1.3. Phytochemical Study

The stored filtrate was used for the various phytochemical and antioxidant studies. A preliminary phytochemical analysis to screen the samples for the presence of phytochemical components such as alkaloids, glycosides, tannins, phenols, saponins and tannins was performed according to the method described by Kokate (1999).

1.3.1. Qualitative Analysis

The qualitative tests were done to find out the presence of the active phytochemical constituents in the defatted extracts (Harborne**,** 1984; Wagner *et al.,* 1984 and Sthal *et al.,* 1965).

Alkaloids (Mayer's test**):** To the extract added 1% HCl and 6 drops of Mayer's reagent were added. An organic yellow precipitate indicated the presence of alkaloids in the sample.

Flavonoids (Lead acetate test**):** The aqueous extract was treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Terpenoids (Salkowski test**):** 10mg of the extract was dissolved in 1ml of

chloroform, 1ml of acetic anhydride was added following the addition of 2ml of conc. H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoides.

Cardiac glycosides (Keller-Killiani test**):** 0.5g of extract diluted to 5ml of water thenadded 2ml of glacial acetic acid containingone drop of ferric chloride solution. This wasunderlayed with 1ml of concentratedsulphuric acid. A brown ring at the interfaceindicates the presence of a deoxysugarcharacteristic of cardenolides. A violet ringmay appear below the brown ring, while in theacetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Phenols (Ferric chloride test**):** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Sterols (Liberman-Burchard's test**):** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Saponins (Froth Test**):** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins.

Tannins (Lead acetate test**):** In a test tube containing about 5ml of an aqueous extract a few drops of % solution of lead acetate was added. A yellow or red precipitate was formed indicating the presence of tannins.

Resins: To 2ml of chloroform extract 5-10ml of acetic anhydride was added, dissolved by gently heating coding and then 0.5ml of sulphuric acid was added. Bright purple colourwas produced. It indicates the presence of resins.

Glycosides: A small amount of alcohol extract samples was dissolved in 1ml water and then aqueous sodium hydroxide solution was added. Formation of a yellow colour indicators the presence of glycosides.

1.3.2. Quantitative Analysis

Determination of Alkaloids (Harborne, 1973): 5g of the sample was weighed in a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to theextract until the precipitation was completed. This solution was allowed to settle and the precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried, weighed and expressed as g/gm extract.

Determination of Flavonoids:Total flavonoid content was estimated by the aluminium chloride colorimetric assay (Zhishen *et al.,* 1999). An aliquot (1ml) of extract and standard solution of Catechin (100mg/ml) was added to 10ml volumetric flask containing 4ml of distilled water. To this 0.3ml of 5% $NaNO_3$ was added. After 5 min, 0.3ml of 10% $AlCl_3$ was added. After 1 min, 2ml of 1M NaOHwas added and the total volume was made up to 10ml with distilled water. The solution was mixed well and the absorbance was measured against reagent blank at 510nm. The value of optical density was used to calculate the total flavonoid content present in the sample.The mean of the three values were expressed as milligrams of Rutin equivalents (mg RE)/ g extract on a dry weight basis.

Determination of Tannins (Van-Burden and Robinson, 1981)**:** 500mg of the sample was weighed into a 50ml conical flask containing 50ml of distilled water and shaken for 1h in a mechanical shaker. Then it was filtered and made up to the mark. From this, 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm within 10 min.

***Determination of Total phenols*:** Total phenolicswere quantified and expressed as gallic acid equivalents according to a method proposed by Singleton *et al.* (1999). About 3.9ml of distilled water and 0.5ml of Folin-ciocalteau reagent were added to 0.1ml of extract in a tube and incubated at room temperature for 3min after which 2ml of 20 % sodium carbonate was added and kept in a boiling water bath for 1min. Phenols react with phosphomolybdic acid in the Folin-ciocalteau reagent in alkaline medium and produce a blue coloured complex (molybdenum blue) that can be estimated colorimetrically at 650nm. The total phenol content of the extract was calculated and expressed as gallic acid equivalent (GAE) mg/ g extract.

1.4. *In-vitro* Antioxidant Activity

1.4.1. Diphenyl-1-picryl hydrazyl (DPPH) method (Blois, 1958)

Various concentrations of sample were mixed with 1ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH (0.2mM). The mixture was shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517nm.

1.4.2. $ABTS^{\bullet+}$ radical cation scavenging activity (Re *et al.,* 1999)

The reaction was initiated by the addition of 1ml of diluted $ABTS^{\bullet+}$ to 10µl of different concentration of methanolic extract of sample and 10µl of methanol as control. The absorbance was read at 734nm and the percentage inhibition was calculated by the following equation.

1.4.3. Chelating effects on ferrous ions/ metal chelating activity (Singh and Rajini, 2004)

The reaction mixture contained 1ml of various concentrations of the extract, 0.1ml of 2mM ferric chloride and 3.7ml of methanol. The control contained all the reaction reagents except sample. The reaction was initiated by the addition of 0.2ml of 5mM ferrozine. After 10 mins at room temperature the absorbance of the mixture was determined at 562nm against blank. A lower absorbance of the reaction mixture was indicated as higher Fe^{2+} chelating ability.

1.4.4. Determination of reducing poweractivity (Yildrin, 2001)

The reaction mixture contained 2.5ml of various concentrations of methanol extract of the sample, 2.5ml of 1% potassium ferricyanide and 2.5ml of 0.2M phosphate buffer (pH 6.6). The control contained all the reagents except the sample. The mixture was incubated at 50°C for 20 minutes and was terminated by the addition of 2.5ml of 10% (w/v) of trichloro acetic acid, followed by centrifugation at 3000rpm for 10 minutes. 5 ml of the supernatant was mixed with 5 ml of deionized water and 1ml of 0.1% Ferric chloride was added. The absorbance was measured at 700nm against blanks that contained distilled water and phosphate buffer. Increased absorbance indicates increased reducing power of the sample. IC_{50} (µg extract/ml) is the effective concentration at which the absorbance was 0.5 reducing power and were obtained by interpolation from linear regression analysis. Ascorbic acid was used for comparison.

1.4.5. Inhibition Concentratin$_{50}$ (IC_{50})

IC_{50} was introduced and interpreted by Brand-Willianms (1995). The discoloration of sample was plotted against the sample concentration in order to calculate the IC_{50} value. It is defined as the amount of sample necessary to decrease the absorbance of the radical by 50%.

2. Results and Disscussion

2.1.1. Preliminary phytochemical analysis

Fruit extract of *T. acuminata* and *S. wightii* were extracted with 4 solvents, viz; peteroleum ether, ethyl acetate, chloroform, ethanol and aqueous and preliminary phytochemical study was undertaken in all extracts to evaluate the presence of active phytoconstituents in fruit part of *T. acuminata* and *S. wightii*. The preliminary phytochemical

qualitative test of extracts confirmed in the presence ofalkaloids, phenols, tannins, glycosides. In fruit part of *S. wightii* resins and steroids are present and in *T.acuminata* cardiac glycosides are present

2.1.2. Total phenolic and flavonoid content in different solvent extracts of fruit *T. acuminata* and *S. wightii.*

The total phenolic content in the different solvent extract offruit part*T. acuminata*measured high when compared to *S. wightii*. Fruit part extract of *T. acuminata* was measured high in acetone (0.80µl) and *S. wightii*fruit part extract was measured high in ethanol (2.44µl) (**Fig. 6**).Highest content of total flavonoids found in fruit part ethyl acetate extract of *T. acuminate* (16.41µl) and fruit part acetone extract of *S. wightii* (29.66µl). The results therefore revealed that fruit part *T. acuminata* and *S. wightii*is a potential source of flavonoids (**Fig. 5**).

2.1.3. *In vitro* Antioxidant Analysis.

DPPH antioxidant activity in fruit part extract of *T. acuminata* and *S. wightii* was accelerated with the increase in the concentrations of the extract from 50-250µg/ml. The petroleum ether extract showed IC_{50} Value 2.70. Ethyl acetate fruit extract showed IC_{50} Value 2.28. Acetone fruit extract showed 5.01 and Ethanol extract showed 3.05 IC_{50} Value. The result confirmed that the fruit part extract of *T. acuminata* has exhibited the highest ability to quench the DPPH radical. When compared to all solvent extract acetone extract showed high activity**.** The IC_{50} Value of the fruit part of the *S. wightii* petroleum ether extract is 2.31. IC_{50} Value of the ethyl acetate extract showed 2.18. Acetone extract showed 5.95 and ethanol extract showed 2.57. The result confirmed that the fruit part extract of *S. wightii* has exhibited the highest ability to quench the DPPH radical when compared to *T. acuminata* fruits (**Fig 9-10**).

The percentage of inhibition of the metal chelation in fruit part extract of *T. acuminata* 67.20% (50 µg/ml) in petroleum ether extract. In the case of ethyl acetate , acetone and ethanol extract the percentage of inhibition of the metal chelation was 36.55% (50 µg/ml) to 40.32% (50 µg/ml), 65.59% (50 µg/ml) and the percentage of inhibition of the metal chelation in fruit part extract of *S. wightii* 69.08% (50 µg/ml) in petroleum ether extract. In the case of ethyl acetate, acetone and ethanol extract the percentage of inhibition of the metal chelation was 65.59% (50 µg/ml) to

9.67% (50 µg/ml), 45.16% (50 µg/ml). *T. acuminata* and *S. wightii* exhibited excellent chelating ability and *S. wightii* fruit exhibited more potential when compared to *T. acuminata* fruit for ferrous ions and might afford protection against oxidative damage (**Fig. 8**).

$ABTS^{\bullet+}$ assay measures the relative antioxidant ability to scavenge the radical ABTS and is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants. Different solvent extracts like petroleum ether, ethyl acetate, acetone and ethanol extracts of the fruit part extract of *T. acuminata* and *S. wightii* were taken to examined for antioxidant activity. $ABTS^{\bullet+}$ radical scavenging activity in fruit part extract of *T. acuminata* (500µg/ml) ethyl acetate extract exhibited the maximum antioxidant activity (89.41%) while ethanol (86.28%) and acetone (83.45%) extracts exhibited moderate activity. Comparatively least activity was exhibited in petroleum ether extract (40.98%).Fruit extract of *S. wightii* in different solvent extracts like petroleum ether, ethyl acetate, acetone and ethanol (97.46%) exhibited high activity when compared other solvent extracts. $ABTS^{\bullet+}$ assay measures *S. wightii* fruits have good antioxidant ability than *T. acuminata* fruits (**Fig. 7).**

The fruit parts of the *S. wightii* extract showed higher reducing power than that of the fruit parts *T. acuminata*.

Fig 5. Total flavonoid content in fruit part of *T. acuminata* and *S. wightii*

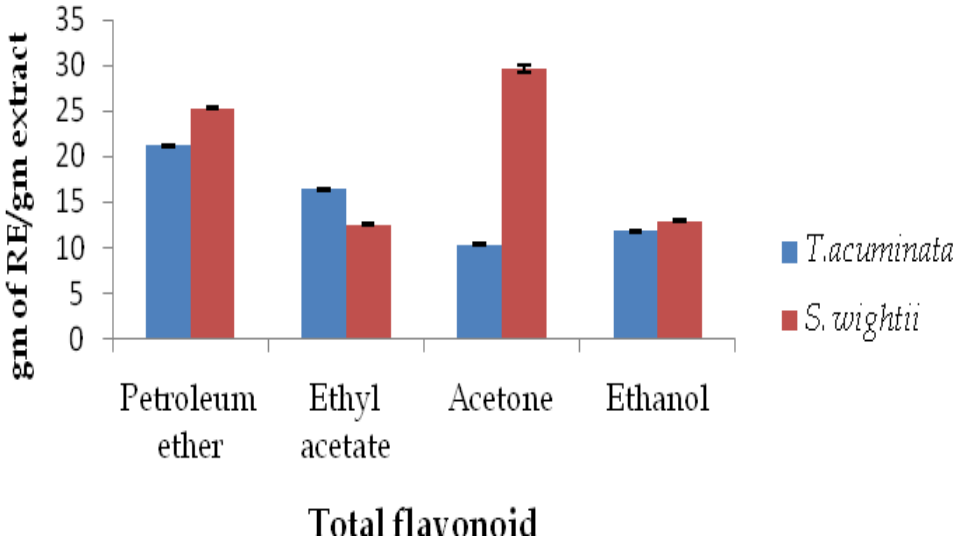


Fig 6. Total phenol content in fruit extract of *T. acuminata* and *S. wightii*

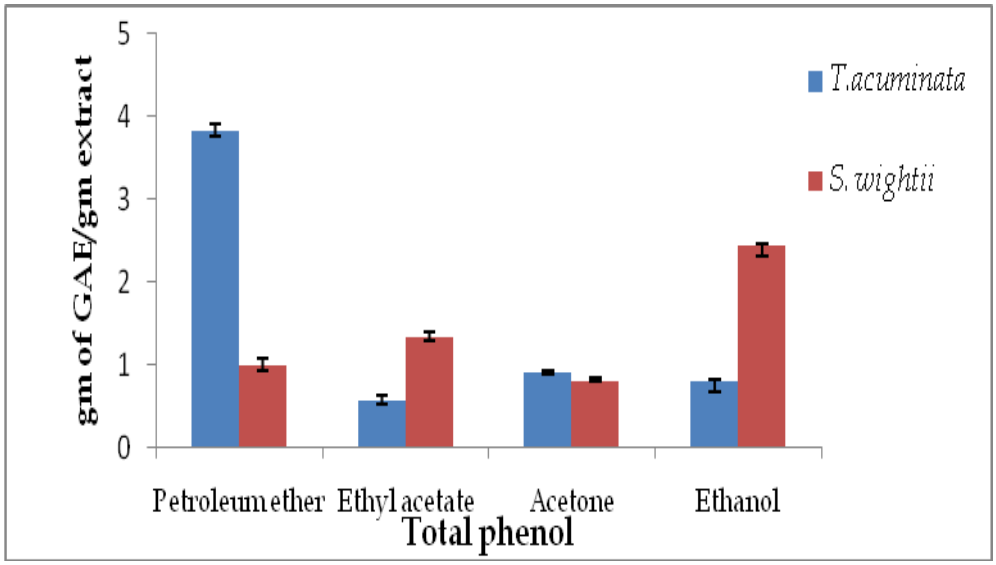


Fig 7. ABTS⁺ radical scavenging activity in fruit part extract of *T. acuminata* and *S. wightii*

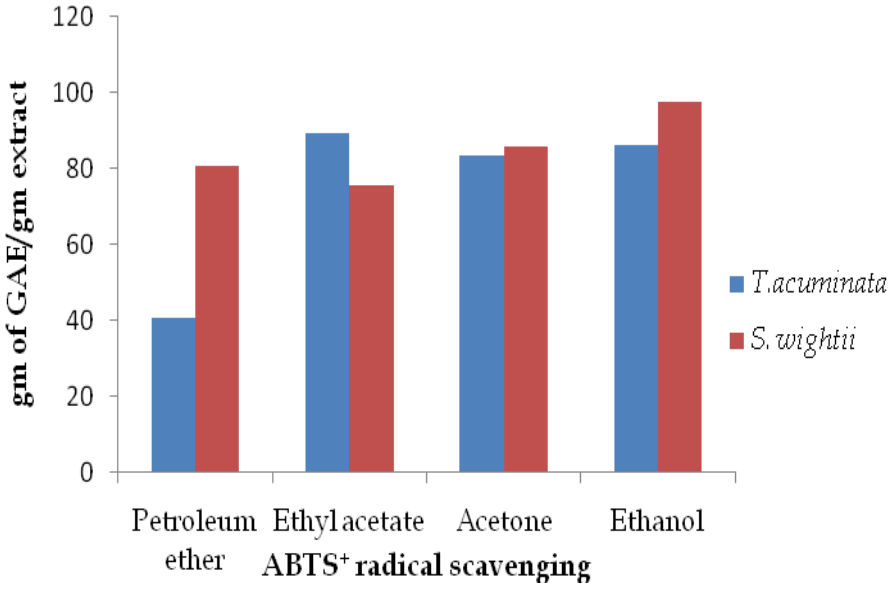


Fig 8. Ferrous iron chelating activity in fruit part extract of *T. acuminata* and *S. wightii*

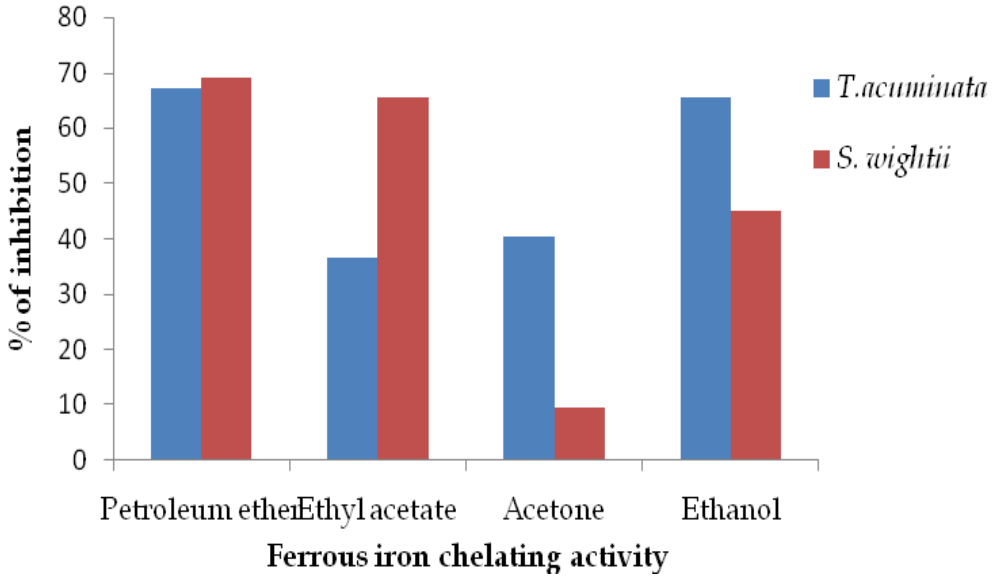


Fig 9. DPPH antioxidant activity in fruit part extract of *S. wightii*

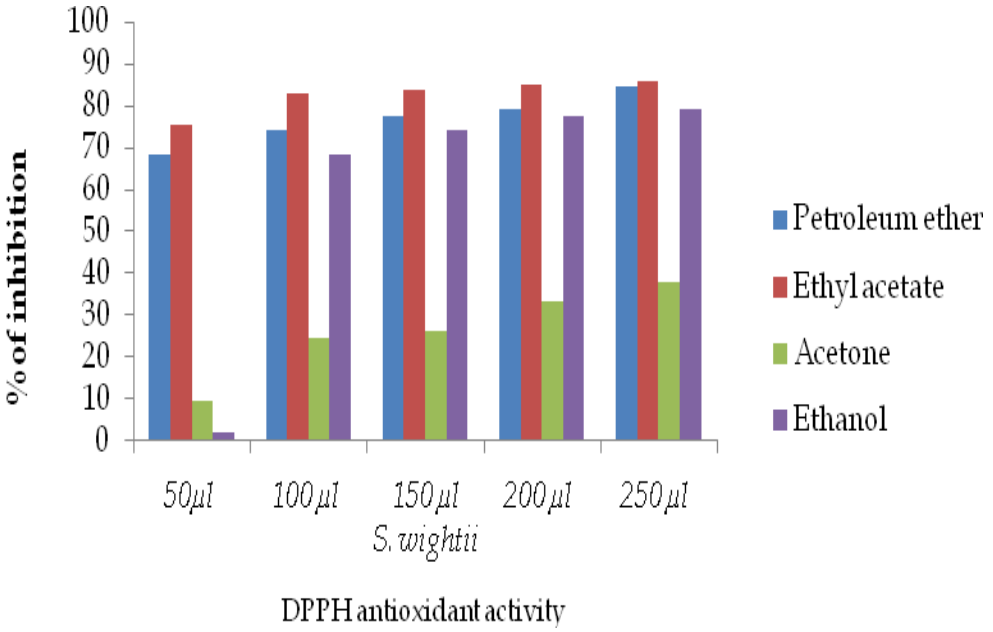
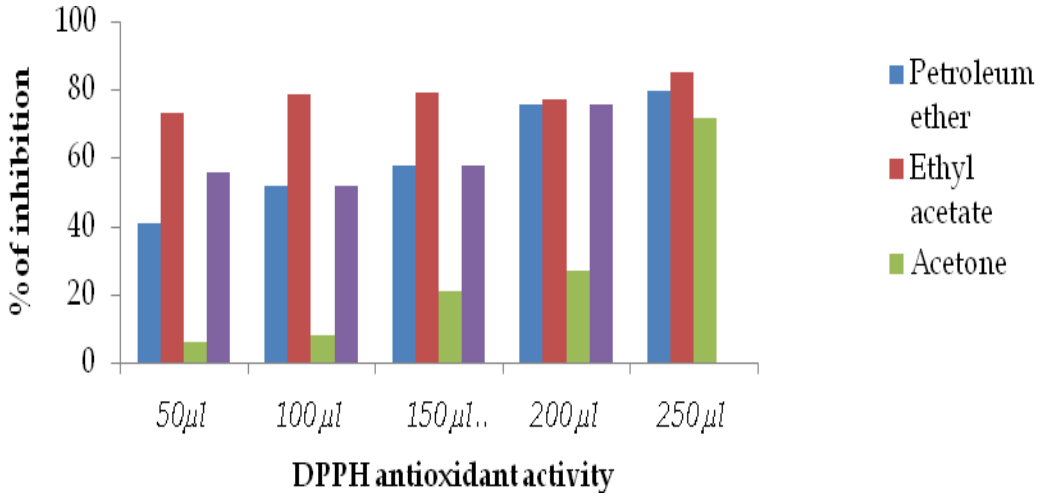


Fig 10. DPPH antioxidant activity in fruit part extract of *T. acuminata*



3. Conclusion

In fruit part of *S. wightii* resins and steroids are present and in *T.acuminata* cardiac glycosides are present. Highest indication of alkaloids, phenols, tannins, glycosides showed in both plant fruit part. From the results obtained for DPPH, $ABTS^{\bullet+}$, ferrous ion chelating assay and reducing power assay of the fruit *T. acuminata* and *S. wightii* can be considered as a potential radical scavenging activity. On the basis of the results it may be concluded that the Fruit of *S. wightii* is more potent than the *T. acuminata* fruit part.

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