

Full Length Research Paper

Identification of bio-active components in leaf extracts of *Aloe vera*, *Ocimum tenuiflorum* (Tulasi) and *Tinospora cordifolia* (Amrutballi)

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Plants are an integral part of life in many indigenous communities. Besides, being the source of food, fodder, fuel, etc., the use of plants as herbal medicines in curing several ailments goes parallel with the human civilization. The present study was carried out to identify the bio-active compounds in various leaf extracts of *Aloe vera*, *Ocimum tenuiflorum* and *Tinospora cordifolia* using different phytochemical screening tests. Total phenolic content (TPC) was determined by Folin-Ciocalteu reagent assay method and total flavonoid content (TFC) using aluminium chloride colorimetric method in the sample extracts. The phytochemical screening results revealed that all the extracts of *A. vera*, *O. tenuiflorum* and *T. cordifolia* are positive for alkaloids, but flavonoids and tannins are present only in some of the extracts. Positive results for saponins and terpenoids are obtained only for *T. cordifolia* extracts. TPC of *A. vera* was high in ethanolic extract (138.13 mg/g) compared to the other two solvents, whereas methanolic extract of *O. tenuiflorum* (114.34 mg/g) and aqueous extract (465.82 mg/g) of *T. cordifolia* exhibited maximum TPC comparatively. TFC of *A. vera* was higher in methanol extract (88.59 mg/g) compared to the other two solvents, whereas methanol (96.34 mg/g) and ethanol (95.46 mg/g) extracts exhibited similar TFC for *O. tenuiflorum* compared to aqueous extract and for *T. cordifolia* ethanol extract (208.36 mg/g), exhibited maximum TFC comparatively. The results of all the three sources were found to be highly significant. However, there is a need to exploit natural sources with medicinal value not only to be used in medical field but also for developing healthy clothing.

Key words: Phytochemical, *Aloe vera*, *Ocimum tenuiflorum*, *Tinospora cordifolia*.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern

drugs have been isolated from natural resources. Traditional medicine is an important source of potentially

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useful new compounds for the development of chemotherapeutic agents. Besides, being the source of food, fodder, fuel, etc., the use of plants as herbal medicines in curing several ailments goes parallel to the human civilization. India is endowed with a rich wealth of medicinal plants, being perhaps the largest producer and rightly acclaimed as the botanical garden of the world (Dubey et al., 2004). Natural products are the source of synthetic and traditional herbal medicine. According to the world health organization (WHO), about 80% of the world's people depend on traditional indigenous medicines, since a large majority of rural people in the developing countries still use these medicines as the first defense in health care (Goleniowski et al., 2006). In India, knowledge about medicinal properties of plants is the basis for their uses as home remedies; plants serve as source of many traditional medicines and continue to provide new remedies to mankind. Indigenous use of medicinal plants all over the world precedes the origin of modern medicine in healthcare system (Aburjai et al., 2007). Certain plant drugs used in modern medicine have ethno-botanical background (Dev, 1997; Fabricant and Farnsworth, 2001). Crude plant extracts (e.g. infusion, tincture, decoction or others) are traditionally used by populations all over the world for medicinal purposes. Although their effectiveness and mechanisms of action have not been scientifically tested in majority of the cases, they often mediate beneficial responses due to their bioactive chemical components (Barnes et al., 2007).

Phytochemical is a natural bioactive compound found in different parts of plants, fruit, flower, stem, leaf and root. Phenolic compounds, cyclic derivatives of benzene with one or more hydroxyl groups associated with the aromatic ring, account for one of the largest and most widely distributed group of phytochemicals (Andjelkovic et al., 2006). They vary considerably in structure with over 8000 naturally-occurring compounds having been identified (Balasundram et al., 2005).

These bioactive compounds work with nutrients and fibers to act as defense system against diseases. Metabolites comprise of alkaloids, terpenoids and phenolic compounds (Krishnaiah et al., 2007) and many more such as flavonoids, tannins, etc.

Aloe vera is a semi tropical plant which belongs to the family Liliaceae, and is used by herbalists for the treatment of different human disorders. *A. vera* contains amino acids, lipids, sterols, tannins and flavonoids. Therefore, this plant is found useful in the treatment of wound, burns, skin disorders and anti-inflammatory activity. A mucilageous gel from the parenchymatous tissue of the leaf has been used for topical treatment of skin burns and wounds (Rovatti and Brennan, 1959). Also often mentioned are the antibacterial, antifungal and even antiviral properties demonstrated by the leaf gel (Klein and Penneys, 1988; Marshall, 1990; Ahmad et al., 1993). As regards to the healing properties, many

researches have demonstrated that the mucilaginous polysaccharides contained in the clear pulp of *A. vera* leaf are the major ingredient responsible for the healing. However, new evidence has shown that emodin, one of the derivatives of anthraquinones produced by superficial pericyclic cells, is also capable of promoting the repair of rats' excisional wounds via stimulating tissue regeneration (Eshun and He, 2004; Tang et al., 2007).

Ocimum tenuiflorum, also called as *Ocimum sanctum* is considered a sacred plant/herb in India, known as "*Tuls*" or "*Tulas*" in Hindi or Holy Basil in English. *Tulasi* belongs to the family Lamiaceae (Labiatae). The plant has yielded different types of constituents including phenol derivatives, flavonoids, phenyl propanoids, triterpenoids (Manitto et al., 1974; Siddiqui et al., 2007a), steroids and steroidal glycosides (Siddiqui et al., 2007b). Wagner et al. (1994) mentioned the use of Basil leaves for a variety of conditions such as catarrhal bronchitis, bronchial asthma, dysentery, dyspepsia, skin diseases, chronic fever, hemorrhage, helminthiasis and topically for ring worms (Singh et al., 1980; Warier, 1995; Kirtikar and Basu, 1993). The oil has antibacterial and antifungal properties (Manitto et al., 1974) and thymol, a constituent of *O. sanctum* is well known as an antiseptic agent (Dikshit and Husain, 1984).

Tinospora cordifolia, known as *Amrutballi* is, a climber plant which belongs to the family Menispermaceae. Extracts of this plant has been shown to possess many therapeutic properties including general tonic, anti-inflammatory, anti-arthritic, anti-malarial, aphrodisiac (Rao et al., 2008), anti-allergic (Nayampalli et al., 1986), anti-diabetic (Wadood et al., 1992), anti-hepatotoxic (Rege et al., 1984) and antipyretic (Kumar and Shrivastav, 1995). The phytochemical constituents in the individual extract have not been specified, it may be possible that multiple constituents of *T. cordifolia* exhibit similar pharmacological properties irrespective of their nature. Although, the active components responsible for therapeutic effects of *T. cordifolia* are not well defined; phenylpropanoid glycosides such as cordifolioside A, cordifolioside B and syringin, have been reported to be main immunomodulatory active components (Maurya et al., 1996; Kapil and Sharma, 1997; Cho et al., 2001).

Natural medicinal plants abundantly available worldwide, are now getting more attention as they possess numerous benefits to society or indeed to all mankind, especially in the field of medicine and pharmacological. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body. The organic compounds usually related with physiological actions on the human body include alkaloids, phenolics, flavonoids, tannins, terpenoids, and steroids (Yadav and Agarwala, 2011).

Therefore, the aim of present study was undertaken to screen various bio-active compounds present in the leaf extracts of *Aloe vera*, *O. tenuiflorum* and *T. cordifolia* and

to evaluate the total phenolic content and total flavonoid content in the plant sources.

MATERIALS AND METHODS

Plant sources

Fresh leaves of *A. vera*, *O. tenuiflorum* and *T. cordifolia* were selected for the study from the University of Agricultural Sciences, Dharwad campus.

Chemicals and reagents

Ethyl alcohol, methanol, chloroform, ammonia, aluminium chloride, hydrochloric acid, sulphuric acid, ferric chloride, sodium hydroxide, sodium chloride, sodium carbonate, sodium nitrite, lead acetate, and gelatin were purchased from Rankem chemicals, Bangalore which were of analytical grade. Folin-Ciocalteu and Dragendorff's reagent was purchased from Merck, Germany. Gallic acid and rutin standards were purchased from Sigma-Aldrich Co. (St. Louis, USA).

Preparation of extracts

The selected plant leaves were cleaned with distilled water and shade dried for a 2 h at room temperature ($25 \pm 2^\circ\text{C}$) to remove the traces of moisture. Two grams of fresh leaf of each sample were weighed, chopped into fine pieces and ground in a pestle and mortar. The ground samples were mixed with 25 ml of each solvent, namely, ethanol, methanol, chloroform and distilled water separately. Incubated under agitation at 200 forward and backward strokes in incubator shaker (Inkarp, Germany) for 24 h at room temperature 25°C . The extracts were centrifuged at 5000 rpm at room temperature (C24 Plus, RemiElektrotechnik, Mumbai) and the supernatants were separated. Residue was re-extracted with 25 ml of the respective solvent and the process was repeated one more time. The supernatants obtained were pooled and the extracts obtained were measured, and filtered using Whatman filter paper No. 40 (125 mm); extracts were stored at 8°C until further analysis within a week.

Phytochemical analysis

The chemical tests described by Ajayi et al. (2011), Raaman (2006) and Rahul et al. (2010) were adopted for the screening of various phytoconstituents like alkaloids, flavonoids, tannins, saponins and terpenoids in the extracts of *A. vera*, *O. tenuiflorum* and *T. cordifolia*.

Test for tannins and phenolic compounds

Ferric chloride test: 1 ml of the extract was separately stirred with 10 ml of distilled water and then filtered. A few drops of 5% FeCl_3 was added to the filtrate. Blue-black or blue-green colouration or precipitation was taken as an indication of the presence of tannins.

Gelatin test: 2 ml of 1% solution of gelatin containing 10% NaCl is added to 1 ml of the extract. White precipitate indicates the presence of phenolic compounds.

Lead acetate test: 3 ml of 10% lead acetate solution was added to 1 ml of the extract. Appearance of bulky white precipitate confirms the presence of phenolic compounds.

Test for flavonoids

Ammonia test: A few drops of 1% NH_3 solution was added to 1 ml of the extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

Sodium hydroxide test: Few drops of 20% NaOH solution was added to 1 ml of the extract. On addition of HCl, the changed yellow colour of the extract turns to a colourless solution that depicted the presence of flavonoids.

Test for alkaloids

Dragendorff test: To 1 ml of the extract, few drops of Dragendorff's reagent were added. A prominent yellow precipitate indicates the positive test.

Wagner test: Few drops of Wagner's reagent were added by the side of the test tube to 1 ml of extract. A reddish-brown precipitate confirms the test as positive.

Mayer test: 1 ml of the extract was stirred with 5 ml of 1% HCL on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with a few drops of Mayer's reagent. The turbidity of the extract filtrate on addition of Mayer's reagent was taken as evidence of the presence of alkaloids in the extract.

Test for saponins

Foam test: About 1 ml of the sample extract was boiled in 20 ml of distilled water in a water bath and filtered; 10 ml of the filtrate was mixed with the 5 ml of distilled water and mixed vigorously for 15 min to form a stable persistent froth. The presence of froth after 5 min was taken as an indication of presence of saponins.

Test for terpenoids

Salkowski test: 1 ml of each extract was mixed with 0.5 ml of chloroform and 1 ml of concentrated H_2SO_4 was carefully added carefully to form a layer. A reddish brown colouration of the interface formed to show positive results for the presence of terpenoids.

Total phenolic content (TPC)

TPC in the extracts was determined by Folin-Ciocalteu assay method (Singleton and Rossi, 1965) with little modification using gallic acid as the reference standard. Briefly, all the solvent extracts were diluted to appropriate volumes and were mixed with 2 ml of 10% Na_2CO_3 solution. Incubated at room temperature for 3 min, 100 μl of Folin-Ciocalteu reagent was added to the mixture. The resulting solution was incubated for 90 min at room temperature under dark, the absorbance was measured at 765 nm using the UV-Vis Spectrophotometer (Varian, Middelburg, Netherlands). The TPC was expressed as gallic acid equivalent (GAE) in milligrams per gram of fresh leaf (Figure 2).

Total flavonoid content (TFC)

TFC was determined by aluminium chloride colorimetric method (Yun et al., 2009; Jyoti et al., 2014) with minor modification. Aliquots (1 ml) of appropriately diluted extracts or standard solutions were pipette into 15 ml polypropylene conical tubes

Table 1. Phytochemical screening of various extracts from selected plant sources.

Chemical screening tests	<i>Aloe vera</i> (Solvent extracts)				<i>O. tenuiflorum</i> (Solvent extracts)				<i>T. cordifolia</i> (Solvent extracts)			
	E*	M*	C*	A*	E*	M*	C*	A*	E	M	C	A*
Alkaloids												
Dragendorff's test	+	+	+	+	+	+	+	+	-	+	+	+
Wagner's test	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids												
Ammonia test	+	+	-	+	-	+	+	+	+	+	-	+
Sodium hydroxide test	+	+	-	-	-	+	-	+	+	+	-	+
Tannins												
Ferric chloride test	-	-	-	-	+	+	+	-	-	+	-	+
Gelatin test	-	-	-	-	-	-	+	+	-	+	+	+
Lead acetate test	+	+	+	+	+	+	+	+	+	+	-	+
Saponins												
Foam test	-	-	-	-	-	-	-	-	-	-	+	-
Terpenoids												
Salkowski test	-	-	-	-	-	-	-	-	+	+	-	+

E*: Ethanol, M*: methanol, C*: chloroform, A*: aqueous, (+) = positive; (-) = negative.

containing 2 ml double distilled H₂O and mixed with 0.15 ml of 5% NaNO₂. After 5 min, 0.15 ml of 10% AlCl₃.6H₂O solution was added and the mixture was allowed to stand for another 5 min, and then 1 ml of 1 M NaOH was added. The reaction solution was well mixed, kept for 15 min and the absorbance was determined at λ 415 nm using the UV-Visible Spectrophotometer (Cary 50, Varian, Middelburg, Netherlands). TFC was calculated using the standard rutin curve and expressed as mg rutin equivalent (mg RE) per gram of fresh leaf (Figure 3).

RESULTS AND DISCUSSION

Yield of extracts

The yield of *Aloe vera* using ethanol and methanol solvents (45 ml of extract/50 ml of solvent) was found to be higher than aqueous (43 ml of extract/50 ml of solvent). *O. tenuiflorum* exhibited higher yield in methanol (41 ml of extract/50 ml of solvent) followed by ethanol (40 ml of extract/50 ml of solvent) and aqueous (39 ml of extract/50 ml of solvent) extracts (Figure 1). On the other hand, aqueous (36 ml of extract/50 ml of solvent) extract of *T. cordifolia* was found to be maximum as compared to methanol (35 ml of extract/50 ml of solvent) and ethanol (32 ml of extract/50 ml of solvent) extracts.

Phytochemical screening

Table 1 records the phytochemical screening of various

extracts of selected species. It is observed from the Table that all the extracts of *A. vera*, *O. tenuiflorum* and *T. cordifolia* exhibited positive results for alkaloids proved by Dragendorff's and Wagner's tests.

The presence of flavonoids was positively proved by both tests (ammonia and sodium hydroxide) in ethanol and methanol extracts of *A. vera*, methanol and aqueous extracts of tulasi and ethanol, methanol and aqueous extracts of tinospora.

All the extracts of *A. vera* and *O. tenuiflorum* depicted the presence of tannins as proved by lead acetate test whereas only ethanol, methanol and aqueous extracts of *T. cordifolia* showed positive results with lead acetate test. However, ethanol, methanol, chloroform extracts of *O. tenuiflorum* and methanol and aqueous extracts of *T. cordifolia* exhibited positive results while chloroform and aqueous extracts of *O. tenuiflorum* and methanol, chloroform and aqueous extracts of *T. cordifolia* depicted the presence of tannins using ferric chloride and gelatin tests, respectively.

None of the extracts of *A. vera* and *O. tenuiflorum* proved the presence of saponins except chloroform extract of *T. cordifolia* using foam test. Similarly, only ethanol, methanol and chloroform extracts of *T. cordifolia* proved the presence of terpenoids with Salkowski test.

Total phenolic content (TPC)

TPC of selected plant sources is described as shown in

Table 2. Total phenolic content (TPC) of the plant leaf extracts.

Extraction solvent	Total phenolic content (GAE* mg/g)		
	<i>Aloe vera</i>	<i>O. tenuiflorum</i>	<i>T. cordifolia</i>
Aqueous	94.42±4.92	80.82±8.63	465.82±23.04
Ethanol	138.13±6.63	113.07±9.81	264.06±18.41
Methanol	95.20±3.23	114.34±11.86	301.42±29.69

GAE*: Gallic acid equivalent.

Table 3. Total flavonoid content (TFC) of the plant leaf extracts.

Extraction solvent	Total phenolic content (RE* mg/g)		
	<i>Aloe vera</i>	<i>O. tenuiflorum</i>	<i>T. cordifolia</i>
Aqueous	72.28±8.70	80.82±7.25	465.82±6.61
Ethanol	138.13±8.57	113.07±4.12	264.06±2.86
Methanol	95.20±8.38	114.34±5.85	301.42±7.59

RE*: Rutin equivalent.

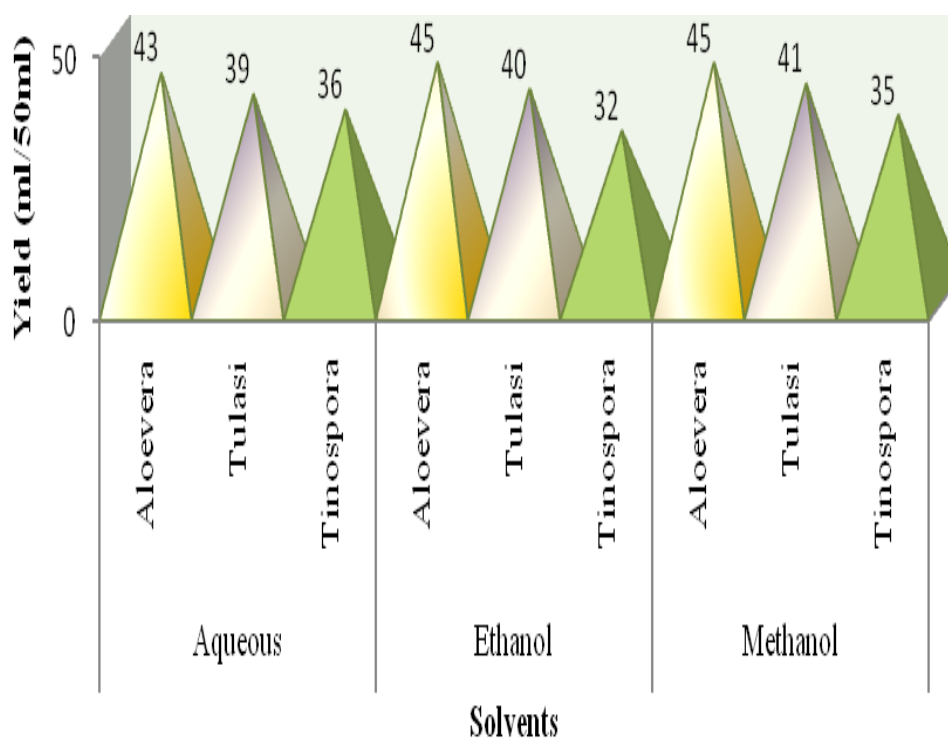
**Figure 1.** Yield of extracts.

Table 2. The results revealed that *Aloe vera* leaves contained higher TPC in ethanol (138.13 mg/g) extract followed by methanol (95.20 mg/g) and aqueous (94.42 mg/g) extracts. On the other hand, methanol (114.34 mg/g) extract of *O. tenuiflorum* yielded higher amount of TPC compared to ethanol (113.07 mg/g) and aqueous (80.82 mg/g) extracts. Further, *T. cordifolia* exhibited maximum TPC using aqueous (465.82 mg/g) against the

phenolic concentration of methanol (301.42 mg/g) and ethanol (264.06 mg/g) solvents.

Total flavonoid content (TFC)

TFC of the plant leaf extracts is described as shown in Table 3. The results revealed that in *A. vera* leaf extracts

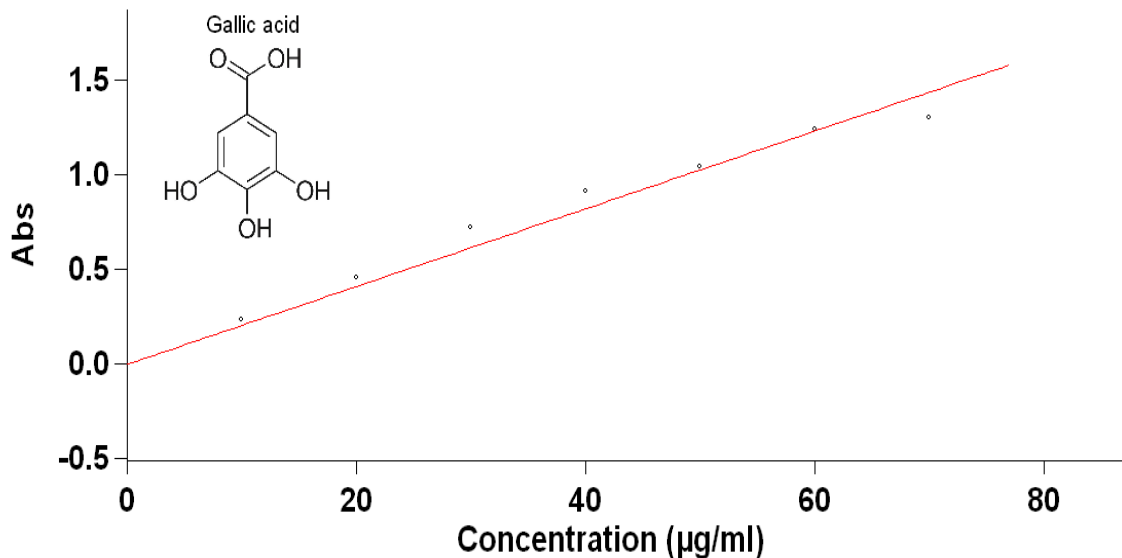


Figure 2. Total phenolic content (TPC): Calibration curve.

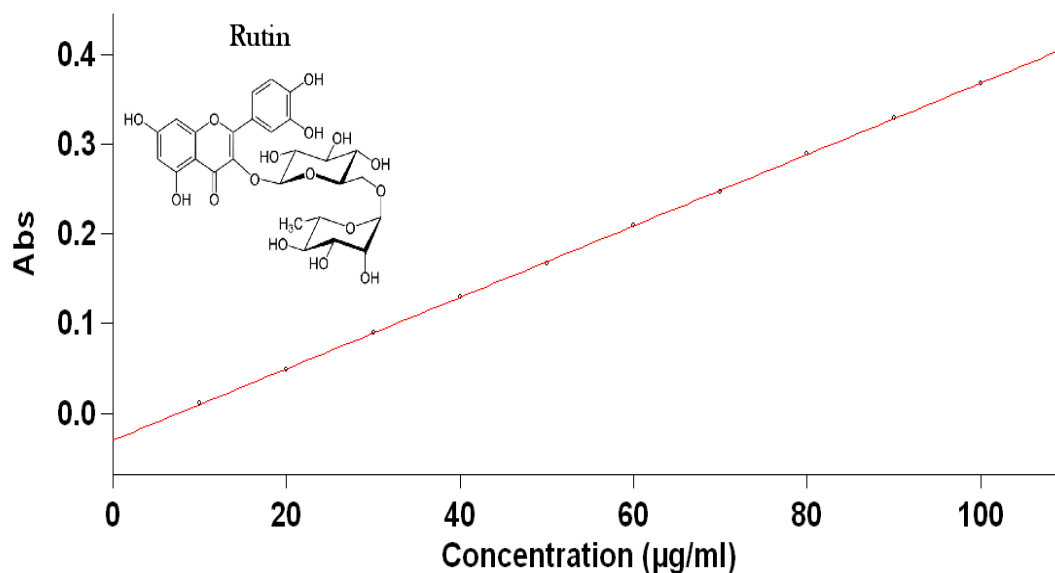


Figure 3. Total flavonoid content (TFC): Calibration curve.

higher TFC was obtained in methanol (88.59 mg/g) followed by ethanol (76.50 mg/g) and aqueous (72.28 mg/g) extracts. Whereas, for *O. tenuiflorum* methanol (96.34 mg/g) and ethanol (95.46 mg/g) extracts exhibited similar TFC with not much significant difference and for aqueous extract it was 61.84 mg/g. For *T. cordifolia*, TFC was higher in ethanol extract (208.36 mg/g) followed by aqueous (178.43 mg/g) and methanol (132.59 mg/g) extracts. However, the treatments of all three sources were found to be highly significant with respect to solvents which imply that there is greater influence of solvents on extraction of various phytoconstituents.

Conclusion

India has a rich flora used in traditional medical treatments; the medicinal properties of these plants could be based on the therapeutic and antioxidant effects of different phytochemicals present in them. The results revealed that alkaloids were found to be present in all the extracts of *A. vera*, *O. tenuiflorum* and *T. cordifolia*. However, flavonoids were present in ethanol, methanol and aqueous extracts of *A. vera* and *T. cordifolia*, and methanol, chloroform and aqueous extracts of *O. tenuiflorum*. Positive results for saponins and terpenoids

were obtained only with *T. cordifolia*. Whereas when compared in between the samples, TPC was high in ethanol extract of *Aloe vera*, methanol extract of *O. tenuiflorum* and aqueous extract of *T. cordifolia*. TFC was high in methanol extract of *A. vera*, methanol and ethanol extracts of *O. tenuiflorum* and ethanol extract of *T. cordifolia*. Furthermore, the results of all the three sources were found to be highly significant. Hence, there is a need to explore the applicability of these plant resources which are rich in phytochemicals/phenolics and may have beneficial effects on health.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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