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Pharmacognostic and *In-vitro* Antioxidant Antimicrobial potentials of Jayanti Veda (*Tridax procumbens* L.)

Research Article

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Abstract

Ayurveda is one of the oldest holistic traditional systems of medicine. *Tridax procumbens* L. known as "Jayanti Veda" which is weed found throughout India. It shows number of pharmacological activities like Hepatoprotective, Anti-inflammatory, Wound healing, Antioxidant, Antimicrobial, Antidiabetic. The present study was carried out to evaluate antioxidant and antimicrobial activity of hydroalcoholic extract and fractions of *Tridax procumbens L.* in order to find possible sources for future novel pharmaceutical formulations. The plant material was subjected for extraction and the extract was further fractionated. Hydroalcoholic extract and fractions were analyzed for different phytocomponents. Fraction 2 was analyzed for phenolic content, Fraction 3 was analyzed for its antimicrobial activity. Fraction 4 was analyzed for its flavonoid content, and antioxidant potentials. The crude extract showed the presence of various phytocompounds whereas F1 contains lipids & waxes. F2 contains phenolics, terpenes & sterols. F3 contains alkaloids. F4 contains flavonoids. The total flavonoid contents of F4 was compared with crude extract (51.19±0.412 and 45±0.073 respectively), antioxidant activity of F4 was compared with crude extract (IC_{50} - 22.7795±0.8208 and 23.247±0.7344 respectively), total phenolic contents of F2 was compared with crude extract (82.24±0.871 and 47.43±0.37 respectively) and antimicrobial activity of F3 [IZD- 1.0cm- (*S. aureus*), 0.9cm (*E.coli*) and crude-0.6cm- (*S, aureus*), 0.5cm (*E.coli*)] Results were found significantly higher for all the fractions as compared to crude extract. The present results revealed that the *Tridax procumbens* L. acts as a powerful antioxidant and antimicrobial agent.

Key Words: *Tridax procumbens* L., DPPH, IZD, Quercetin, Ascorbic acid, Antioxidant, Antimicrobial assay.

Introduction

Ayurvedic medicines and Herbal formulations including herbs, herbal materials, herbal preparations, and finished herbal products, has become an increasingly important part of the global healthcare system. Phytochemical and pharmacological studies revealed that one herb may contain hundreds, or thousands, of chemical components that exerted pharmacological effects via a network of various targets and pathways (1).

Tridax procumbens L., often known as "coat buttons", belongs to *Asteraceae* family. Plant bears daisy like yellow centered white or yellow flowers with three toothed ray florets. The leaves are serrated and arrowhead-shaped. The fruit is a rigid achene with stiff hairs on one end and a feathery, plume-like white pappus on the other end (2).

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Antioxidants protect cells from the harmful effects of free radical molecules like reactive oxygen free radicals (ROS), reactive hydroxyl radicals (OH), superoxide anion radicals (O2), hydrogen peroxides (H2O2) and peroxyl (ROO) produced during the normal metabolic process of oxidation and produce metabolic products that target lipids in cell membranes or DNA. Thus, including DNA damage, carcinogenesis, and cellular degeneration, as well as heart disease and arthritis (3, 4).

Plants with an aromatic ring containing one or more hydroxyl groups are known as phenolic compounds. There are over 8000 naturally occurring plant phenolics, with flavonoids accounting for about half of them. Phenolics have a wide range of biochemical properties, including antioxidant, antimutagenic, and anticarcinogenic properties, as well as the potential to modify gene expression. Phenolics are the most abundant phytochemicals in plants and plant products, accounting for the majority of antioxidant action.

Flavonoids are the most abundant group of naturally occurring phenolic chemicals, found in both free form and as glycosides in various plant parts. They exhibit antimicrobial, mitochondrial adhesion inhibition, antiulcer, anti-arthritic, anti-angiogenic, anticancer, protein kinase inhibition, and other

biological actions. Flavones and flavanols are common forms of phenolics. Flavonoids are antioxidants that protect against cardiovascular disease, certain types of cancer, and cell component degeneration. Because of their polyphenolic composition, they can scavenge harmful free radicals such as super oxide and hydroxyl radicals (5).

This study deals with the evaluation of Pharmacognostic parameters, antioxidant and antimicrobial activity of *Tridax procumbens* L. extract and its various fractions. The results were compared between crude extract and its fractions.

Materials and methods

Materials

All the chemicals, reagents and TLC plates were purchased from Sigma-Aldrich Ltd.

Methods

Collection and processing of plant material

The plant material was collected randomly from local areas of Belagavi Karnataka (India) and authenticated by ICMR Belagavi. (Accession number RMRC-1577). Then the plant material was washed, air dried, homogenized to fine powder and stored in airtight container until further use.

Pharmacognostic studies

Macroscopic study

The freshly collected plant material was subjected for macroscopic evaluation various characters like shape size colour odor taste surface characters and texture were observed. (Fig.1)

Physicochemical parameters

Powdered plant material was examined for various physicochemical parameters like loss on drying, total ash, water soluble ash, acid insoluble ash, and extractive value as per WHO guidelines (Table 1) (6).

Powder microscopy

The powder microscopy was carried out using Trinocular microscope as per standard procedure and their specific diagnostic characters were recorded (Fig. 2) (7,8).

Extraction of plant

100 gm of powdered plant material was subjected for maceration using alcohol and water (70:25). Mark was further extracted by Soxhlet method using alcohol as a solvent and extract is fractionated using suitable method described by P. Cos et al. Various fractions obtained are F_1 (Pet. Ether fraction for lipids waxes) F_2 (Methanol fraction for phenolics, terpenes and sterols) F_3 (dichloromethane fraction for alkaloids) F_4 (Water fraction for salts and hydrophilic substances) % Yield is depicted in (Table 2) (9).

Phytochemical investigation of extract and fractions

Extract and fractions (F_1, F_2, F_3, F_4) were screened for various phytochemicals such alkaloids, glycosides, flavonoids, tannins, saponins, steroids, and triterpenes as per standard procedure. (Table 3) (6, 10).

Spectroscopic analysis

Spectroscopic analysis was performed using an UV-Spectrophotometer at wavelength ranging from 200nm to 800nm for crude extract and its fractions (Fig 3 & Table 4) (11).

Chromatographic analysis

Thin layer chromatography (TLC)

By phytochemical screening and UV spectroscopy analysis it is confirmed that, crude extract and fraction 4 (F_4) contains flavonoid. Thus, crude extract and fraction 4 were taken for further TLC study. Thin layer chromatographic study was performed as per standard procedure using Quercetin as a standard marker on silica gel 60 F254 plates and mobile phase used was Toluene: Ethyl acetate: Formic acid (7:3:0.5) using aluminum chloride reagent as detecting agent (Fig4 Table5) (12).

Total flavonoid content by $AlCl_3$method

Total flavonoid content of crude extract and fraction 4 (F_4) was analyzed using aluminum chloride colorimetric method with slight modification according to Chang et al. Quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in ethanol 95% and diluted to 2-10µg/mL. 1 mL of each concentration of standard solutions, as well as 1 mL of each sample solution, were mixed with 3 mL ethanol 95%, 0.2 mL of aluminum chloride 10%, 0.2mL 1 M Potassium acetate and 5.6mL of distilled water was added. The mixture was incubated at room temperature for 15 min with intermittent shaking. The absorbance was measured at 376 nm against a blank using SICAN 2301UV-spectrophotometer. Total flavonoid was calculated as mean ± SD (n = 3) and expressed as weight of quercetin equivalent (QE) per 1g of dry extract and fraction (Fig5 Table 6) (13, 14).

Total Phenolic content

The total phenolic content of the crude extract and fraction 2 (F2) was analyzed using the Folin-Ciocalteu reagent, as described by *Pourmorad et al*, with minor modifications. Gallic acid was used as a standard to plot a calibration curve. 10 mg Gallic acid was dissolved in 10 mL methanol and diluted to 2-10µg/mL. 2.5 mL Folin–Ciocalteu and 2.5 mL distilled water were diluted with 0.5 mL of standards and samples. After 5-minutes incubation period, 2 mL of aqueous sodium carbonate solution (7.5 % w/v) was added. After shaking the final mixture, it was incubated at room temperature for 15 minutes in the dark. The absorbance of all standards and samples were measured at 765 nm using SICAN 2301 UV-spectrophotometer. Total phenolics was calculated as mean ± SD (n = 3) and expressed as weight of gallic acid equivalent (GAE) per 1g of dry extract and fraction (Fig6 Table 7) (13,15,16).

Antioxidant activity

The radical scavenging activity of crude extract and fraction 4 (F4) against the DPPH radical was determined according to standard procedure with slight modifications (18). 3 mL of 0.1 mM DPPH radical solution was diluted with 3 mL of methanolic solutions of crude extract and fraction 4 (concentration of 10-50µg/mL). After 30 min incubation in the dark, absorbance of the mixture was measured at 515 nm. The colour of the solution changed from violet to light yellow during reduction by the antioxidant. The absorbance of blank solution was measured at 515nm using 3 mL methanol and 3 mL 0.1 mM DPPH radical solution. As a positive control, quercetin was used. The experiment was repeated three times. The activity of radical scavenging was determined using the following formula (15, 16).

$$\%\ Radical\ scavenging\ activity = \frac{|control|-|sample|}{|control|} \text{X } 100$$

The 50% inhibitory concentration (IC50) was calculated as the amount of extract required to react with half of the DPPH radicals (Fig 7 Table 8).

Antimicrobial activity

In vitro antimicrobial activity was assessed by using Agar Well Diffusion method against *Staphylococcus aureus* and *Escherichia coli*, which are the types of gram positive and gram negative organisms respectively. The crude extract, fractions (F1, F2, F3, F4) and the control (1000µg/ml in DMSO) were placed into wells bored with a sterile borer. To allow for prediffusion of the extracts into the agar, plates were kept in the refrigerator for two hours. The plates were then incubated at 37°C for 24 hours. The antimicrobial activity was determined by measuring the diameter of zone of inhibition (IZD) (Fig8 A, B&Table9) (18, 19, 20).

Results

Pharmacognostic studies

Macroscopic study

Jayanti Veda (*Tridax procumbens* L.) is a flowering plant, widespread weed and pest plant. It is an annual, sometimes perennial prostrate to ascending herb with dark green leaves having lanceolate and ovate shape. Roots are brown in colour. Plant has the characteristic odor with acrid taste. Leaves are 3-7 cm long, 1-5 cm wide, roots are 15-32 cm long.

Fig: 1. Jayanti Veda whole plant

Physicochemical parameters

Physicochemical screening was carried out to utilize the quality of selected medicinal plant *Tridax procumbens* L. Quality control parameters such as loss on drying, total ash, acid-insoluble ash, water soluble ash, alcohol and water-soluble extractive matter were studied. The total ash of crude powder was 4.8%, water soluble ash was 2.14% and acid insoluble ash was 3.05%. Less amount of these three parameters indicate that the inorganic matter and silica was less in *Tridax procumbens* L. The extractive value of crude powder was maximum in water (13.2%) followed by methanol (7.17%) and minimum was in petroleum ether (6.2%).

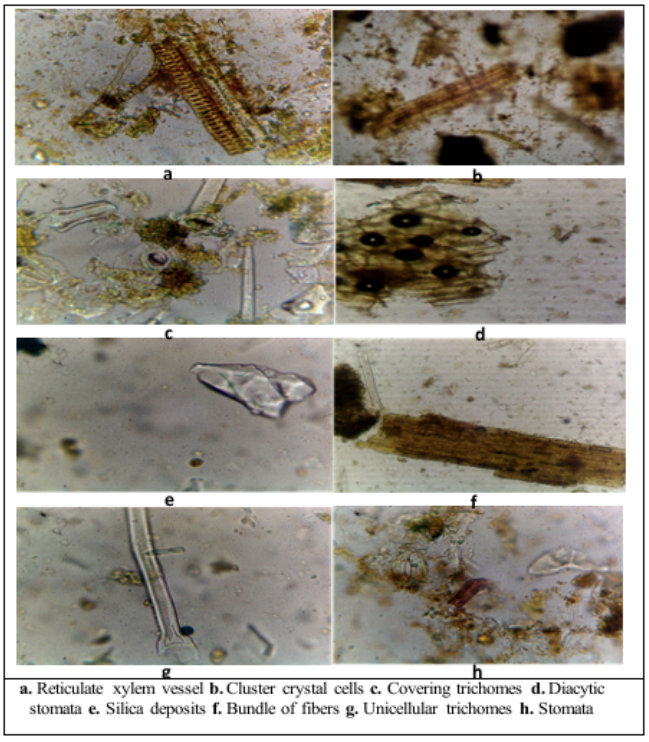
Table 1: Physicochemical parameters

Parameters		Values
LOD		8.30%
Ash value	Total ash	4.8%(w/w)
	Acid insoluble ash	3.05%(w/w)
	Water soluble ash	2.14%(w/w)
Extractive value	Ethanol	7.17%(w/w)
	Petroleum ether	6.2%(w/w)
	Water	13.2%(w/w)

Powder microscopy

The powder microscopy reveals the presence of different types cell constituents like trichomes, reticulate xylem vessel, cluster crystal cells, covering trichomes, diacytic stomata, silica deposits, bundle of fibers, unicellular trichomes, stomata.

Fig: 2. Microscopic features of powder of *Tridax procumbens* L.



a. Reticulate xylem vessel **b.** Cluster crystal cells **c.** Covering trichomes **d.** Diacytic stomata **e.** Silica deposits **f.** Bundle of fibers **g.** Unicellular trichomes **h.** Stomata

Plant extracts preparation:

After the extraction the crude extract obtained was subjected for fractionation. The yield and consistency of

extract and various fractions (F_1, F_2, F_3, F_4,) were tabulated in Table 2.

Table 2: Yield, consistency, color of extract & various fractions

Extracts	Yield %	Consistency	Color
Crude extract	16.6	Sticky	Light brown
Fraction 1	7	Sticky	Light green
Fraction 2	6.8	Sticky	Light green
Fraction 3	5.3	Sticky	Light green
Fraction 4	11.2	Sticky	Light brown

Phytochemical investigation

Phytochemical investigation revealed the presence of various phytocompounds like carbohydrates, alkaloids, steroids, flavonoids, triterpenes, tannins, saponins in the extract and fractions.

Table 3: Phytochemical investigation

Sl. No	Phyto-compounds	Status				
		Crude	F1	F2	F3	F4
1	*Carbohydrates*	+	-	-	-	-
2	*Alkaloids*	+	-	-	+	-
3	*Steroids*	+	+	+	-	-
4	*Flavonoids*	+	-	-	-	+
5	*Lipids*	+	+	+	-	-
6	*Triterpenes*	+	+	+	-	-
7	*Tannins*	+	-	-	-	+
8	*Saponins*	+	-	-	-	+

Spectroscopic analysis

Crude extract and fractions of *Tridax procumbence* was analyzed under UV range of 200-800nm and the profile showed the peaks at different wavelength indicating the various phytoconstituents.

Table 4: UV-VIS Spectroscopic analysis

Sample	Peaks (nm)
Crude	663,605,535,504,409,372,317,302,290,263,228
F1	651,524,472,408,264,236
F2	4,64,34,32,84,234
F3	2,52,234
F4	7,41,66,32,83,223

Chromatographic analysis

Thin layer chromatography (TLC)

TLC estimation of crude extract showed 9spots with Rf values 0.13, 0.24, 0.31, 0.50, 0.59, 0.62, 0.69, 0.75, 0.96. Fraction4 (F_4) showed 7 spots with Rf values 0.12, 0.28, 0.49, 0.62, 0.78, 0.85, 0.95. Standard Quercetin with Rf value 0.49. (Table 6 &Fig 4). TLC estimation revealed the presence of flavonoid compound Quercetin in both extract and fraction 4 by comparing the Rf value 0.49.

Fig: 3. UV-VIS Spectrum of Extract and Fractions

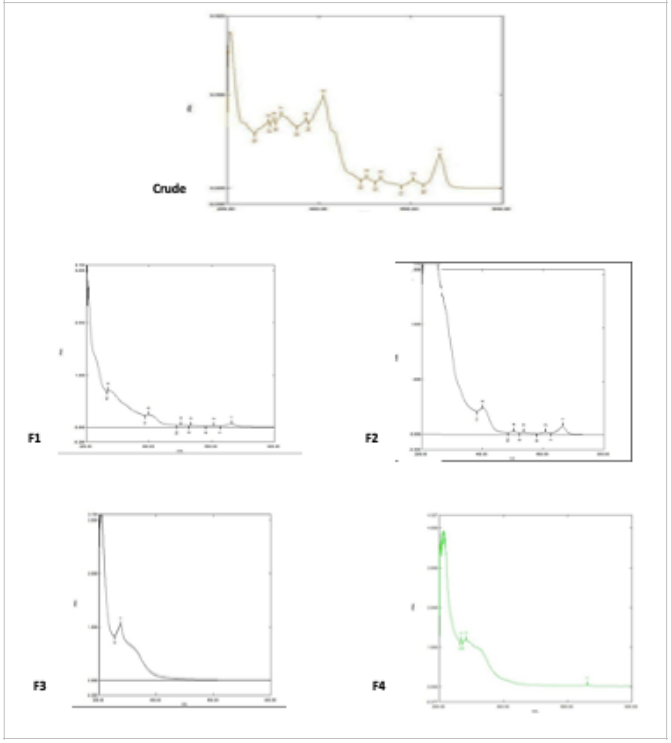


Table 5: Retention Factor values of TLC

Sample	No of spots	Rf values
Crude	9	0.13,0.24,0.31,0.49,0.59,0.62, 0.69,0.75,0.96
Standard Quercetin	1	0.49
Fraction 4	7	0.12,0.28,0.49,0.62,0.78, 0.85,0.95

Fig: 4. Thin Layer Chromatography

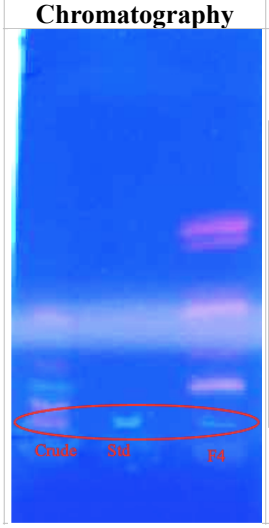
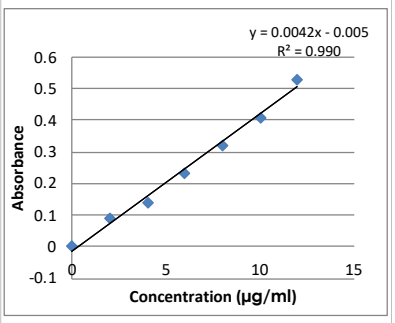


Fig: 5. Standard Calibration of Quercetin



Total flavonoid content

Both extract and fraction 4 (F4) are rich in flavonoid compounds, the results of total flavonoid and quercetin concentration in the extract of *Tridax procumbens* L. was found to be 45 mg QE/g DW and F4 was 51.19 mg QE/g DW. The calibration curve for standard Quercetin with regression equation and R2 value was y = 0.0042x - 0.005R^2 = 0.990.

Total Phenolic content

Both extract and Fraction 2 are rich with phenolic compounds, the results of total phenolic and Gallic acid concentration in the extract of *Tridax procumbens* L. was found to be 47.43 mg GAE/g DW and F2 was 82.24 mg GAE/g DW. The calibration curve for standard Gallic acid with regression equation and R2 value was y = 0.0042x + 0.02 R^2 = 0.992.

Fig: 6. Standard Calibration of Gallic acid

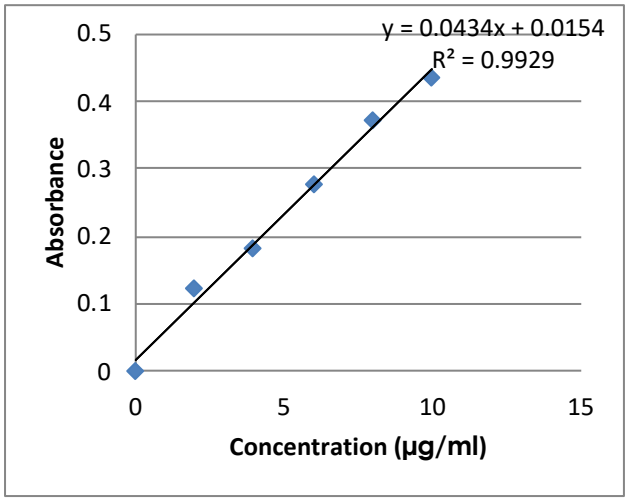


Fig: 7. Standard Calibration of DPPH

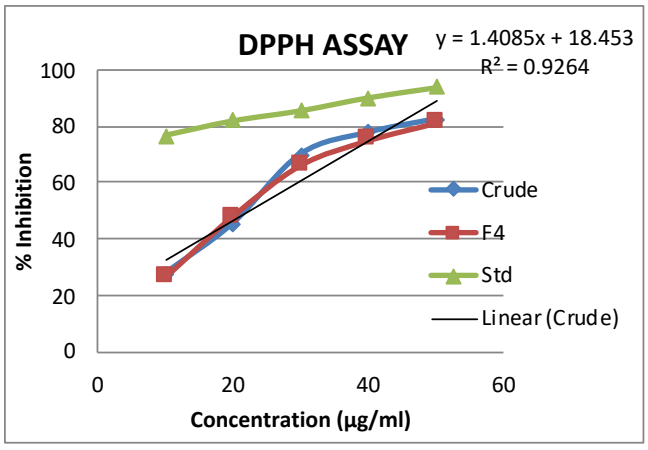


Table 6: Total flavonoid and phenolic content

Samples	Conc. (µg/ml)	TFC (mg QC/g DW)	TPC (mg GAE/g DW)
Crude	1000	45	47.43
F2	1000	-	82.24
F4	1000	51.19	-

Antioxidant activity

Fraction 4 showed higher Antioxidant activity (IC_{50}22.7795±0.8208µg/ml) than the crude extract (IC_{50}23.347±0.7344µg/ml). These IC_{50} values are compared with standard Quercetin (18.0707±3.607µg/ml). The calibration curve for standard DPPH with regression equation, $R^2$and IC_{50}value of extract and fraction has been depicted in Fig 7 Table 8.

Table 7: Antioxidant activity

Sl. No.	% Inhibition			
	Conc (µg/ml)	Standard (Ascorbic acid)	Extract	F4
1	10	76.68	27.98	26.42
2	20	81.86	45.59	48.19
3	30	85.49	69.95	66.32
4	40	90.15	77.64	75.13
5	50	93.78	82.38	81.35
IC_{50} (µg/ml)		18.0707± 3.607	23.347± 0.7344	22.7795± 0.8208

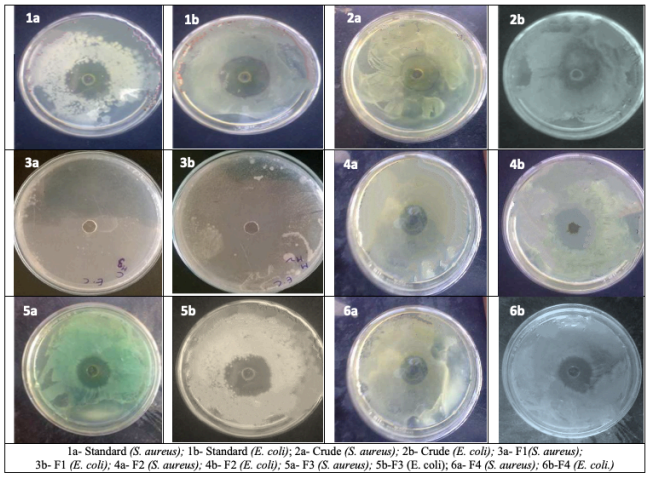
Antimicrobial activity

The results of antimicrobial activity of *Tridax procumbens* L. showed in the table 9. The extracts & fractions showed significant zone of inhibition against selected bacterial species viz., *S. aureus and E. coli.* Fraction 3 showed favorable antimicrobial activity as compared to crude extract.

Table 8: Zone of inhibition of Extract & Fractions

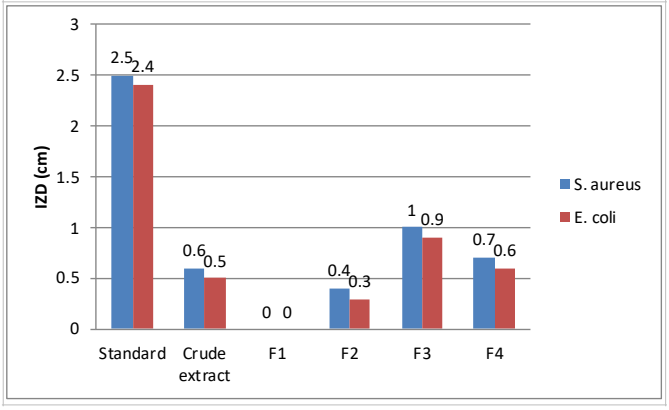
Sl No	Sample	Zone of inhibition (cm)	
		***S. Aureus* (a)**	***E. coli* (b)**
1	Standard (ciprofloxacin)	2.5	2.4
2	Crude	0.6	0.5
3	F1	0.0	0.0
4	F2	0.4	0.3
5	F3	1.0	0.9
6	F4	0.7	0.6

Figure: 8 A. Zone of inhibition of Extract & Fractions



1a- Standard *(S. aureus)*; 1b- Standard *(E. coli)*; 2a- Crude *(S. aureus)*; 2b- Crude *(E. coli)*; 3a- F1*(S. aureus)*; 3b- F1 *(E. coli)*; 4a- F2 *(S. aureus)*; 4b- F2 *(E. coli)*; 5a- F3 *(S. aureus)*; 5b-F3 (E. coli); 6a- F4 *(S. aureus)*; 6b-F4 *(E. coli.)*

Figure 8 B: Effect of Extract & Fractions on *S. aureus* and *E. coli*



Discussion

Tridax procumbens L. is a well known plant that has been used as medicinal plant for diverse pharmacological activity. To study the effect of *Tridax procumbens* L. on antioxidant and antimicrobial activity, the plant material was collected, processed in to powder and it was examined for various physicochemical, phytochemical, antioxidant and antimicrobial parameters Powered material was extracted by maceration using alcohol and water (70:25) as solvent followed by Soxhlet extraction. To ensure the quality and purity of *Tridax procumbens* L. extract & fractions on antioxidant and antimicrobial activity associated with the amounts of bioactive compounds in it, the physicochemical analysis has been conducted. Extract and its fractions showed values that satisfy the requirements for extracts quality and purity. To ensure the presence of specific phytocompounds in the fractions, TLC & spectroscopic analysis was conducted, which confirmed the presence of phenolics in F2 and flavonoids in F4 fraction. Total phenolic and total flavonoid represent the total amounts for phenolic and flavonoid compounds in the extracts. Total phenolic and total flavonoid content of F2 and F4 was higher (82.24±0.871 mg GAE/g DW and 51.19±0.412 mg QE/ g DW, respectively) than the crude extract. Antioxidant ability of the F4 was higher with IC_{50} (22.7795±0.8208 µg/mL) than the crude extract. Antimicrobial potential of F3 was higher than the crude extract for both *S. aureus and E. coli* bacterial species (IZD- 1.0cm & 0.9cm, respectively). This study confirmed that the antioxidant and antimicrobial activity of *Tridax procumbens* L. were attributed to the values of total phenolic, total flavonoid and IZD. Thus, the development of *Tridax procumbens* L. to be source of herbal medicine for oxidation and microbial was suggested.

Conclusion

The hydroalcoholic extract and its various fractions significantly showed flavonoids and phenolic content with antioxidant and antimicrobial activity. The study reflects on the comparative antioxidant and antimicrobial potential between crude extract and its fractions. Remarkably, fraction 3 showed good antimicrobial activity and fraction 4 showed good antioxidant potentials as compared to hydroalcoholic extract of *Tridax procumbens* L.

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References

1. Dahiyat F-EL, Rashrash M, Abuhamdah S, Abu Farha R, Babar ZU. Herbal medicines. a cross-sectional study to evaluate the prevalence and predictors of use among Jordanian adults. *J. Pharm. Policy Pract.* 2020 Dec; 13(1):1-9.
2. Kumar S, Prasad A, Iyer SV, Vaidya S. Pharmacognostical, phytochemical and pharmacological review on *Tridax procumbens L. Linn*. *Int. j. pharm. biol.* 2012; 3(4):747-51.
3. Habila JD, Bello IA, Dzikwi AA, Musa H, Abubakar N. Total phenolics and antioxidant activity of *Tridax procumbens L. Linn*. *Afr. J. Pharm. Pharmacol*. 2010 Mar 31; 4(3):123-6.
4. Jalalpure SS, Ghagane SC, Puranik SI, Kumbar VM, Nerli RB, et al. *In vitro* antioxidant and anticancer activity of *Leea indica* leaf extracts on human prostate cancer cell lines. *Integr. Med. Res.* 2017 Mar 1; 6(1):79-87.
5. Sulaiman CT, Balachandran I. Total phenolics and total flavonoids in selected Indian medicinal plants. *Indian J. Pharm. Sci.* 2012 May; 74(3):258.
6. Zambare AV, Chakraborthy GS, Banerjee SK. Pharmacognostic studies of potential herb *Tridax procumbens Linn*. *Int J Pharm Sci Res*. 2010; 1(9):58-62.
7. Khandelwal K. Practical pharmacognosy. Pragati Books Pvt. Ltd.; 2008 Sep 7.
8. Pande J, Padalia H, Donga S, Chanda S. Pharmacognostic, physicochemical and phytochemical studies of *Andrographis echioides Nees* and *Tridax procumbens L*. leaf and stem. *Pharma Innov J*. 2018; 7(6):303-15.
9. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. *J Ethnopharmacol.* 2006 Jul 19; 106(3):290-302.
10. Kuldeep G, Pathak AK. Pharmacognostic and Phytochemical Evaluation of *Tridax procumbens Linn*. *J. pharmacogn. phytochem.* 2013 Jan 1; 1(5).
11. Jain PK, Soni A, Jain P, Bhawsar J. Phytochemical analysis of *Mentha spicata* plant extract using UV-VIS, FTIR and GC/MS technique. *J Chem Pharm Res*. 2016;8(2):1-6.
12. Dougnon G, Ito M. Medicinal uses, thin-layer chromatography and high-performance liquid chromatography profiles of plant species from Abomey-Calavi and Dantokpa Market in the Republic of Benin. *J. Nat. Med.* 2020 Jan;74 (1): 311-22.
13. Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pak J Pharm Sci*. 2009 Jul 1; 22(3):277-81.
14. Sulastri E, Zubair MS, Anas NI, Abidin S, Hardani R, et al. Total phenolic, total flavonoid, quercetin content and antioxidant activity of standardized extract of *Moringa oleifera* leaf from regions with different elevation. *Pharmacogn. J.* 2018; 10(6s).
15. Wong-Paz JE, Contreras-Esquivel JC, Rodríguez-Herrera R, Carrillo-Inungaray ML, López LI, et al. Total phenolic content, *in vitro* antioxidant activity and chemical composition of plant extracts from semiarid Mexican region. *Asian Pac. J. Trop. Med.* 2015 Feb 1; 8(2):104-11.
16. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents

of some selected Iranian medicinal plants. *Afr. J. Biotechnol.* 2006; 5(11).

17. Brandão LB, Santos LL, Martins RL, Rodrigues AB, Rabelo ED, et al. Larvicidal Evaluation against Aedes aegypti and Antioxidant and Cytotoxic Potential of the Essential Oil of *Tridax procumbens L*. Leaves. *Sci. World J.* 2021 Jan 1; 2021.
18. Pandey S, Seth A, Tiwari R, Singh S, Behl HM, et al. Development and evaluation of antimicrobial herbal cosmetic preparation. *Afr. J. Pharm. Pharmacol.* 2014 May 29;8(20):514-28.
19. Chavan CB, Shinde UV, Hogade M, Bhinge S. Screening of *in-vitro* antibacterial assay of *Barleriaproinitis LINN. J. Herb. Med. Toxicol.* 2010; 4:197-200.
20. Jalalpure SS, Agrawal N, Patil MB, Chimkode R, Tripathi A. Antimicrobial and wound healing activities of leaves of *Alternanthera sessilis Linn. Int. J. Green Pharm.* 2008; 2(3).

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