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RESEARCH ARTICLE

EFFECT OF UNDER CONVENTIONAL SOLVENT EXTRACTION ON POLYPHENOL CONCENTRATIONS AND ANTIOXIDANT ACTIVITIES IN *Thespesia lampas.*

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Key words:-
Antioxidant activity, extraction solvents, IC_{50}, *Thespesia lampas*, total phenolic content.

Abstract

Objectives: This study aimed to evaluate the polyphenol content and antioxidant activity in dried leaf, fruit, root and flower of *Thespesia lampas* Dalz. and Gibs (Cav.) extracted in acetone, isopropanol, acetonitrile and dicholoromethane (under conventional solvents).
Material and methods: The antioxidant profile was evaluated using total antioxidant capacity (TAC), ABTS and DPPH assay, Fe^{2+} chelating ability, iron reducing power (RP) *in vitro* assays.
Results: Highest total phenolic content (TPC) and flavonoid were noted in acetone extracted flower (Fac) and dichloromethane extracted root (Rdcm) respectively. Fac showed maximal activity for TAC (212.16 µM TE/ mg DW), ABTS (IC_{50} = 1.12 ± 0.00 $mgmL^{-1}$), DPPH (IC_{50} = 0.87 ± 0.01 $mgmL^{-1}$) and RP (IC_{50} = 67.48 ± 0.54 $mgmL^{-1}$) while Rdcm showed highest Fe^{2+} chelating ability (IC_{50} = 1.70 ± 0.05 $mgmL^{-1}$). Acetone emerged as the most efficient solvent in extraction of antioxidants among the solvents exercised. A positive correlation was observed between TPC and ABTS (R^2 = 0.952), TPC and TAC (R^2 = 0.881), ABTS and TAC (R^2 = 0.892); and DPPH and RP (R^2 = 0.688) assays.
Conclusions: Efficiency of under conventional solvents for extraction of potent organs of *Thespesia lampas* leading to higher antioxidant activity as a promising source.

Introduction:-

Traditional knowledge on the utilization of medicinal plants is the guidelines for their therapeutic properties. There are several of medicinal plants that have been practiced as folklore medicines from thousands of years but remain ignored of scientific investigations. *Thespesia lampas* is among such plants species which is poorly explored for its medicinal properties. *Thespesia lampas* Dalz. and Gibs (Cav.) commonly known as Jungli Bhindi, Ran Bhindi, Van Kapas, is a tall green glabrous undershub species of Malvaceae family. It is generally found along the hill slopes of forests in India and Eastern Tropical Africa (Nadkarni, 2007). T. lampas had been reported for its number of pharmacological activities. Leaf extracts have showed potential treatment against skin problems, inflammation and ringworm infections (Patil, 2003). Stem fractions have antidiuretic and antidysentric properties (Adhikari et al., 2007). Root reported to be useful in antihyperlipidemic (Sangameswarm, 2008a), antidiabetic (Jayakar and Sangameswarm, 2008), anti lypoxigenase (Kumaraswam and Satish, 2008), antihelmintic (Satish and Ravindra, 2009), antioxidant (Kumaraswam and Satish, 2008, Sangameswarm et al., 2009), anti microbial (Vasaraj et al.,

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1997), hepatoprotective activity (Sangameswarm, 2008b, Stephen et al., 2012). Presence of active biomolecules such as as quercetin, gossypol (Luckefahr and Fryxell, 1967), ellagic acid, tannic acid and rutin (Stephen et al., 2012)) suggest the curative actions against several diseases caused by free radicals.

Free radicals are released as byproducts in series of physiological mechanism in human body. Over production of free radical may trigger the generation of oxidative stress to bio-organelles leading to diseased condition such as cardiovascular, atherosclerosis, cancer (Halliwell, 1994), degenerative diseases (Shahidi et al., 1992), inflammatory diseases (Sreejayan and Rao, 1996), Alzheimer's disease (Di Matteo and Esposito, 2003). Such harmful sequels of free radicals could be balanced by antioxidants leading to healthy life.

Therefore the purpose of this study was to investigate total phenol content, total flavonoid content and antioxidant activity in acetone, isopropanol, acetonitrile and dicholromethanol extracts of leaf, fruit, stem, root, flower of *T. lampas* based on total antioxidant capacity, ABTS free cation decolorization assay, DPPH free radical scavenging assay, Ferrous metal ion chelating ability, iron reducing power.

Material and methods:-

2.1 Chemicals and reagents

Folin Ciocalteau reagent, ABTS (Azinobis ethylbenzothiazoline 6-sulphonic acid), DPPH (1,1-diphenyl-2-picrylhydrazyl, α-tocopherol), Trolox (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were purchased from Himedia (India). Any other chemicals were of analytical grade.

2.2 Plant materials

The samples of leaf, stem, fruit, root and flower of *T. lampas* were collected from Karencho-Dhareshwar mount of Vijaynagar forest region, North Gujarat, India in 2014. The voucher herbarium specimen SN-13/BSJO was deposited in Arid Zone Regional Center, Jodhpur, Rajasthan, Botanical Survey of India, for taxonomic identification. The samples collected were cleaned with milliQ water (merck) and air dried in shade at room temperature for seven days. Samples were grind to fine powder in blender.

2.3 Preparation of Extracts

Approximately 500 mg of ground sample was extracted separately into 50 mL of acetone, isopropanol, acetonitrile, dicholoromethane twice. The suspensions were stirred for overnight in dark at 37 °C followed by ultrasonication for 10 min. Further, combined extracts were centrifuged at 10,000 rpm and evaporated under vacuum to reduce the volume up to 50 mL. The supernatants were filtered and stored at 4 °C to be analyzed within a week.

2.4 Total phenolic content (TPC)

Total phenolic content in the leaf, stem, fruit, root and flower extracts of *T. lampas* was were assessed as described by Singleton and Rossi (1965). Briefly, 2.50 mL of extract was diluted with 2.25 mL of H_2O and 250 µL Folin-Ciocalteu's reagent and allowed to stand for 5 min. This mixture was neutralized by 7% (w/v) Na_2CO_3 and kept at in dark for 90 min. Absorbance was measured at 765 nm using UV-VIS spectrophotometer Quantification of total phenolic content was done on the basis of standard curve of gallic acid (20-100 µg mL^{-1}, $y = 0.002 + 0.019$, R^2=0.989) and results were expressed in mg gallic acid equivalents per g of dry weight (mg GAE/g DW).

2.5 Total flavonoid content (TFC)

Total flavanoid content in extracts were determined according to Chang et al., (2002). Aliquot of 0.5 mL of sample extract was mixed with 1.5 mL of H_2O, 0.5 mL $AlCl_3$ (10% w/v), 0.1 mL of 1M potassium acetate and diluted with 2.8 mL H_2O. The solution was incubated for 30 min absorption measured at 415 nm using against blank. TFC was quantified on the basis of calibration curve of qurecetin (1-10 µg mL^{-1}, $y = 0.090x - 0.0095$, R^2=9911) and results were expressed in mg qurecetin equivalent per g dry weight (mg QE/g DW).

2.6 Total antioxidant capacity (TAC)

TAC of extracts was evaluated as described by Prieto et al., (1999). Extracts was combined with 1 mL of reagent solution (0.3 N sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a boiling water bath for 90 min. Then, samples were cooled to room temperature and the absorbance was measured at 695 nm against blank. Antioxidant capacity was expressed as µg α-tocopherol equivalent per mg of dry weight of plant (µM TE/ mg DW).

2.7 ABTS free radical (ABTS •+) decolorization assay
Radical scavenging activity was performed by improved ABTS method (Cai et al., 2004). Briefly, 7.0 mM ABTS and 2.45 mM $K_2S_2O_8$ mixed for the production of ABTS•+ and kept in dark for 16 h at room temperature. ABTS•+ solution was diluted with distilled water till the absorbance obtained 0.700 (±0.05) at 734 nm. For sample analysis 3.90 mL of diluted ABTS•+ solution was added to 100 µL of extracts and allowed to stand for 6 min in dark .Decrease in absorbance over time was monitored at 734 nm. Trolox (50- 400 µM,$y = 0.192x - 7.637$, $R^2 = 0.999$). The percentage of ABTS•+ scavenged was calculated by equation 1:

Radical scavenging activity $\% = [(A_0 - A_1)/A_0]$ x100

Where, A_0 is the absorbance of control (without test sample), A_1 is the absorbance of reaction mixture (with test sample).

2.8 DPPH free radical (DPPH•) scavenging assay
The extracts were analyzed in terms of hydrogen-donating or radical-scavenging ability using the stable radical DPPH (Blois, 1958). Briefly, the reaction mixture contained 100 µL of different extracts and 2 mL 0.1 mM DPPH solution. The decreased levels of absorbance of reaction mixture were measure at 517 nm against the blank. Vitamin C was used as the positive control ($y = 0.694x - 0.713$, $R^2 = 0.999$). The percent DPPH decolonization of the sample was calculated according to equation 1.

2.9 Ferrous metal ion chelating ability
The chelating activity of extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis et al. (1994). Extract (0.5 mL), 1.6 mL of deionized water, 0.05 mL of 2 mM $FeCl_2$ were added to 0.1 mL ferrozine (5 mM). The iron chelating activity of extract was calculated using the equation 1. EDTA (10-60 µg mL^{-1}) was used for standard curve preparation ($y = 1.675x + 4.933$, $R^2 = 0.989$).

2.10 Iron reducing power (RP) assay
The iron reducing power of the extract was calculated according to the method given by Oyaizu (1986). Briefly, the reaction mixture contained 500 µL extracts (0.5-2 mg/mL), 500 µL of 0.2 M sodium phosphate buffer (pH 6.6) and 500 µL of 1% (w/v) potassium ferricyanide (III) and incubated at 50 ◦C for 20 min. After cooling at room temperature, 500 µL of trichloroacetic acid (10% w/v) was added and centrifuged for 10 min at 1000 x g. To 500 µL supernatant, 500 µL of distilled water and 100 µL of iron (III) chloride were added. After 10 min absorbance was measured at 700 nm against distilled water as a blank. Higher absorbance of reaction mixture indicates higher reducing power. Reducing power of samples were quantified on vitamin C based calibration curve (100-100 $^{mgL-1}$, $y = x + 0.731$, $R^2 = 0.978$).

2.11 Statistical analysis
All experiments were carried out in triplicate. Data were presented as means ±standard deviation. The dose–response curve was obtained by plotting the percentage inhibition vs. concentration. The concentration giving 50% inhibition (IC_{50}) was calculated by linear interpolation of graph. One-way ANOVA followed by Dunnett's Test ($\alpha = 0.05$) was used to compare any significant differences between IC_{50} values of extracts for ABTS, DPPH, chelating ability and iron reducing power with the positive control. Data of TPC, TFC and TAC assays were analyzed by Multiple comparisons of means using Tukey's Multiple Range Test ($p < 0.05$) with Prism GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, CA, USA). Correlation between IC_{50} values with TPC and TFC was calculated by Pearson'scorrelation coefficient. The significance level was $\alpha = 0.05$. The data was subjected for principal component analysis (PCA) to identify possible grouping of analyzed solvent extracts of source material using IBM SPSS version 21 (SPSS Inc. 1989e1996, Chicago, IL, USA).

Results and discussion:-
In the present study, polyphenolics including total phenol and flavonoid content as well as antioxidant activities were studied in leaf, fruit, root and flower samples extracted in acetone, isopropanol, acetonitrile and dichloromethane. The choice of diverse extraction solvents was based on the polarity as a major separation technique to extract structurally different groups of phenolic compounds according to their solubility in solvents.

3.1 Total phenolic and flavonoid content
Results showed that TPC and TFC of the source matrices varied significantly as function of solvent used for extraction. Root extract and flower extract of *T. lampas* exhibit significantly ($p < 0.01$) higher amount of TPC

(149.12 mg GAE/g DW and 127.34 mg GAE/g DW respectively) as compared to leaf (12.46 mg GAE/g DW) and fruit (3.09 mg GAE/g DW) extracts (Fig. 1A). The TPC value in *T. lampas* extracts ranged from 281.25 mg GAE/g DW of Fdcm to 0.87 mg GAE/g DW of FRip. Flower (281.25 mg GAE/g DW) and root (210.25 mg GAE/g DW) extracted in acetone showed significantly ($p < 0.001$) higher TPC than that of isopropanol, acetonitrile and dichloromethane (Fig. 1 B). TFC was found highest in flower extract of *T. lampas* (15.63 mg QE/g DW) followed by root extract (13.88 mg QE/g DW), leaf extract (9.13 mg QE/g DW) and fruit extract (3.53 mg QE/g DW). It significantly varied ($p < 0.01$) among fruit, root and flower extracts (Fig. 1 C). Dichloromethane extracts of both root and flower exhibited highest amount of TFC (24.91 mg QE/g DW and 24.55 mg QE/g DW) respectively (Fig. 1 D). TPC value were found to be significant in flower and fruit extracts of the plant and it decreased in following order of solvent system among root and flower extracts : acetone > isopropanol > acetonitrile > dichloromethane (Fig. 1 A and 1 B). Root and flower of *T. lampas* possibly may have apotic charged phenolic compounds which were more soluble in acetone and solubility of extracted phenolic matrices gradually decreased in pure polar and nonpolar solvents). Among solvents, dichloromethane depicted significant variation in root and flower extracts for TFC. This may caused by the presence of non polar composition in flavonoid make-up of plant matrix. The choice of solvents system for extraction TFC found to be non significant in both leaf and fruit extracts (Fig. 1 D).

3.2 Antioxidant activities

The antioxidant capacity of *T. lampas* extracts were evaluated through formation of a green phosphate/Mo5+ complex. TAC was found highest in roots and flower extracts (95.12 and 93.33 µM TE/ mg DW) of the plant followed by leaf (60.16 µM TE/ mg DW) and fruit (14.54 µM TE/ mg DW) extracts (Fig. 1 E). Fac showed highest amount of TAC (212.16 µM TE/ mg DW) (Fig. 1 F). The root extract of the species showed highest TAC antioxidant activity. TAC varied significantly ($p < 0.001$) among the solvents system of flower extract and decreased in following order: acetone (212.16 µM TE/ mg DW) < isopropnanol (103 µM TE/ mg DW) < acetonitrile (35 µM TE/ mg DW) < dicholoromethane (30.33 µM TE/ mg DW) (Fig. 1 F). In leaf, fruit and root extracts there were no significant variation noted among selected solvents. Extracts of fruit were comparatively least efficient. IC_{50} is the concentration of extract at which the 50 % of inhibition is achieved. A lower IC_{50} value corresponds to a higher antioxidant activity. The antioxidant potential of extracts to scavenge the ABTS•+ was evaluated in relation to the scavenging activity of trolox. IC_{50} value for ABTS•+ scavenging activity was strongest in extract of Fac (IC_{50}= 1.12 $mgmL^{-1}$) followed by Rac (IC_{50}= 1.51 $mgmL^{-1}$), Ran (IC_{50}= 2.90 $mgmL^{-1}$), Rip (IC_{50}= 3.11 $mgmL^{-1}$) and Fip (IC_{50}= 3.51 $mgmL^{-1}$) while the extracts of FRac (IC_{50}= 39.98 $mgmL^{-1}$), Ldcm (IC_{50}= 24.69 $mgmL^{-1}$) and FRdcm (IC_{50}= 20.89 $mgmL^{-1}$) were dramatically inactive (Table 1). Regardless of solvents used in fruit extraction, extracts of fruit were poor scavengers of ABTS•+. No activity was noted in fruit extracts of isopropanol (Frip) and acetonitrile (Fran) for maximum detection limit of two $mgmL^{-1}$ (Table 1). Dicholoromethane extracts of all the parts was least efficient solvent for ABTS•+. Although flower acetone extract exhibited maximal ABTS•+ scavenging activity (lowest IC_{50} value) but on an average, IC_{50} value of *T. lampas* for root extracts in all the solvents accounted to be most efficient. The DPPH activity was evaluated by ability of plant extract to donate electron or hydrogen which turns the nitrogen centered violet free radical DPPH solution to yellow (Mathew and Abraham, 2004). DPPH• scavenging activity was highest in Fac extract (IC_{50}= 0.87 $mgmL^{-1}$) with slight increase in Rac (IC_{50}= 1.39 $mgmL^{-1}$) followed by Ldcm (IC_{50}= 1.59 $mgmL^{-1}$) and Lan extracts (IC_{50}= 1.70 $mgmL^{-1}$) (Table 1). Fruit extracts of isopropanol, acetonitrile and dichloromethane showed reduced DPPH• scavenging activity. Flower extracts showed most reduced activity (higher IC_{50} value) and was not detectable at maximum concentration of two $mgmL^{-1}$ in Fan and Fdcm (Table 1). Kumaraswamy and Satish reported IC_{50} (72.28 μgmL^{-1}) of aqueous root extract of *T. lampas* for DPPH• scavenging activity (Kumaraswam and Satish, 2008). The differences with the previous record may attribute to the extraction procedures, experimental dose designing, climatic variations and harvesting times. Ferrozine reacted with the Fe^{2+} to form Fe^{2+}–Ferrozine, a stable magenta complex absorbance measured at 562 nm. The generation of chelated ferrous ion at 50% concentration of EDTA was found to be 0.02 $mgmL^{-1}$ in iron chelating activity. It is clear from Table 1 that leaf, fruit and root extracted in dichloromethane were better chelators of Fe^{2+} as compared to rest of the examined extracts. The iron reducing power of the plant is a direct indicator of its antioxidant activity. The reducing property of the extract serves as a hydrogen atom donor and reduce Fe^{3+} ferricyanide complex to the green shades of Fe^{2+}. Extract of Fac had the most potent reducing power (IC_{50} = 67.48 $mgmL^{-1}$) (Table 1) and acetone served as powerful reductones with increased activity among rest of the solvents. Fruit extracts depicted the weakest reducing power as compared to other extracts. There was a significant impact of extraction solvents on the extraction of polyphenol concentrations and antioxidant activities in leaf, fruit, root and flower organs of *Thespesia* but despite of solvent treatment, root extracts (Rac, Rip, Ran and Rdcm) verified as a most potent organ of the species with lowest average IC_{50} values (Table 1). The antioxidant activities of the plant basically attributed to the direct or indirect interaction of plant with various environmental stresses such as altitude, sunlight, UV-radiation, temperature and soil factors that triggers accumulation of these phytochemicals (Rawat et al., 2011, Nath et al., 2017).

3.3 Correlation among the total phenolic, flavonoid content and antioxidant activities
The correlation analysis indicated that TPC was strongly and positively correlated with both ABTS and TAC (R^2 = 0.952, R^2 = 0.881 respectively). Similarly, ABTS and TAC (R^2 = 0.892) as well as DPPH and RP (R^2 = 0.688) were positively correlated to each other (Table 2). The quantified values of TPC, TAC and ABTS as well as DPPH and RP are comparable as they are closely related to each other with strong positive correlation (Table 2). The positive correlation suggested larger contribution of phenolic portion compounds to the overall antioxidant activity. It is in accordance with other several reports where TPC was found to be positively correlated with ABTS assay (Rawat et al., 2011). The polyphenolics of plant matrices may act cooperatively, antagonistically or synergistically with other components present in crude extracts (Ni´ciforovi´c et al., 2010).

The datasets were further explained by principal component analysis which clearly suggested the total variance among 47.69 %, 21.76 % and 15.69 % in the first, second and third components (Table 3). The principal component analysis explained the classification of the values in different groups according to their characteristic phytochemicals and antioxidant activities. Eigenvalues value of analyzed data sets recommended three components of the datasets with total variability of 47.69 %, 21.76 % and 15.69 % respectively (Table 3). Total of four possible groups were identifiable in the rotated space plot on the basis of similarities and differences among all the analysed extracts (Fig. 2 A). Fac, Rac, Rip, Fip and Ran were grouped together in TPC-TAC-ABTS indicating positive PC1 score (Fig. 2 B). Rdcm and Fdcm positioned in TFC group with negative PC 2 score where as DPPH-RP and FeCHLT groups were poorly separated by PC 1 and PC 2. Group DPPH-RP and TFC were better explained by PC 1 and PC 3 plot (Fig. 2 C) where, Frac, Lac, Lan, Fan, Fran and Lip grouped in DPPH-RP scoring negative both PC1 and PC 3 and Ldcm, Frdcm, Frip lied in TFC group with positive PC 3 score.

Conclusions:-

The present study represents the first report to carry out a comprehensive study in *T. lampas* leaf, fruit, root and flower extracted in under-conventional extraction solvents. The choice of solvents of diverse polarity was kept in centre of the study for better quantification of total phenolic, total flavonoid and antioxidant activities in *T. lampas*. Acetone could be suggested as most efficient solvent among the solvents employed for extraction of total phenolic, TAC, ABTS, DPPH activities and reducing power while total flavonoid and Fe chelating ability were best extracted in dichloromethane. Root and fruit of the plant were more potent organs in terms of activities studied. Thus, *T. lampas* may serve as a potent source of naturally occurring antioxidants which need to be more explored for its phytoconstituents and chemical composition suggesting a wide array of the biological and pharmacological activities.

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Table 1:- IC $_{50}$ value of antioxidant activities in *Thespesia lampas* extracted in under conventional solvents.

Parts	Sample	IC $_{50}$ (mgmL^{-1})			
		ABTS	DPPH	Fe2+ chelating ability	Iron Reducing Power
Leaf	Acetone (Lac)	6.66±0.57^{cd}	2.86±0.00^{fg}	7.56±1.89^{bc}	291.61±1.67^{ghi}
	Isopropanol (Lip)	7.15±1.05^{cd}	2.10±0.00^{gh}	5.50±0.25^{cde}	426.96±3.53^{ef}
	Acetonitrile (Lan)	13.31±1.10^{bc}	1.70±0.03^{hi}	6.18±0.12^{bcde}	509.98±7.31^{de}
	Dicholoromethane (Ldcm)	24.69±5.87^{b}	1.59±0.07^{hi}	2.61±0.16^{ef}	540.52±2.68^{d}
	Average	12.95±1.86	2.06±0.02	5.46±0.60	442.27±10.30
Fruit	Acetone (FRac)	39.98±8.79^{a}	3.51±0.02^{ef}	6.23±3.31^{bcde}	842.78±7.08^{c}
	Isopropanol (FRip)	ND	18.53±0.06^{a}	3.45±0.06^{def}	934.21±1.34^{bc}
	Acetonitrile (FRan)	ND	13.36±0.82^{c}	7.48±0.42^{bc}	942.87±0.01^{b}

	Dicholoromethane (FRdcm)	20.89±13.71^{b}	15.36 ±0.02^{b}	2.92±0.53^{ef}	1392.43±1.36^{a}
	Average	15.22±5.62	12.69±0.01	5.02±0.81	1028.07±12.90
Root	Acetone (Rac)	1.51±0.14^{d}	1.39±0.02^{hi}	5.54±0.44^{cde}	113.94±5.89^{kl}
	Isopropanol (Rip)	3.11±0.07^{cd}	2.67±0.05^{fg}	7.12±0.30^{bcd}	310.54±8.17^{gh}
	Acetonitrile (Ran)	2.90±0.01^{cd}	1.97±0.15^{gh}	5.36±0.33^{cdef}	197.22±4.90^{ijk}
	Dicholoromethane (Rdcm)	6.62±1.08^{cd}	3.51±0.51^{ef}	1.70±0.05^{f}	184.46±0.98^{jk}
	Average	3.54±0.32	2.39±0.18	4.93±0.12	201.54±2.04
Flower	Acetone (Fac)	1.12±0.00^{d}	0.87±0.01^{i}	9.52±1.67^{b}	67.48±0.54^{l}
	Isopropanol (Fip)	3.51±0.74^{cd}	2.17±0.02^{gh}	15.16±3.82^{a}	253.25±2.56^{hij}
	Acetonitrile (Fan)	4.07±0.23^{cd}	3.76±0.00^{e}	ND	355.89±8.94^{fg}
	Dicholoromethane (Fdcm)	19.08±0.24^{b}	5.28±0.70^{d}	ND	524.30±6.70^{d}
	Average	6.95±0.31	3.02±0.18	6.17±1.37	300.23±4.68
Trolox		0.15±0.00	-	-	-
Vitamin C		-	0.07±0.00	-	120.34±2.70
EDTA		-	-	0.02±0.00	-

Data represented as mean ± standard deviation. N = 3. ND: Not detected. Values followed by the different letter under the same column are significantly different by Duncan's multiple comparison test ($p < 0.05$).

Table 2:- Pearson's correlation coefficients of antioxidant activity, IC_{50}, total phenolics and total flavonoids content for quantitative determinations in the selected parts of *T. lampas* included in this investigation.

Assay	TPC	TFC	TAC	ABTS	DPPH	Fe Chelating Activity	Iron Reducing Power
TPC	1	0.426	0.881**	0.952**	0.171	-0.134	0.469
TFC	0.426	1	0.451	0.254	-0.059	0.090	0.134
TAC	0.881**	0.451	1	0.892**	0.149	-0.099	0.233
ABTS	0.952	0.254	0.892**	1	0.205	-0.237	0.442
DPPH	0.171	-0.059	0.149	0.205	1	0.074	0.688**
Fe Chelating Activity	-0.134	0.090	-0.099	-0.237	0.074	1	0.112
Iron Reducing Power	0.469	0.134	0.233	0.442	0.688**	0.112	1

TPC— Total phenol content, TFC— Total flavonoid content, TAC— Total antioxidant capacity, ABTS— ABTS free radical scavenging assay, DPPH— DPPH free radical scavenging assay. **Significant $p < 0.01$.

Table 3:- Total variance explained using principal components based on phytochemicals and antioxidant activities in *T. lampas*.

S.No.	Component Initial Eigenvalues				Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %		Total	% of Variance	Cumulative %
1	3.33	47.69	47.69		3.33	47.69	47.69
2	1.52	21.76	69.45		1.52	21.76	69.45
3	1.09	15.69	85.15		1.09	15.69	85.15
4	0.63	9.00	94.16				
5	0.34	4.85	99.01				
6	0.05	0.71	99.73				
7	0.01	0.26	100.00				

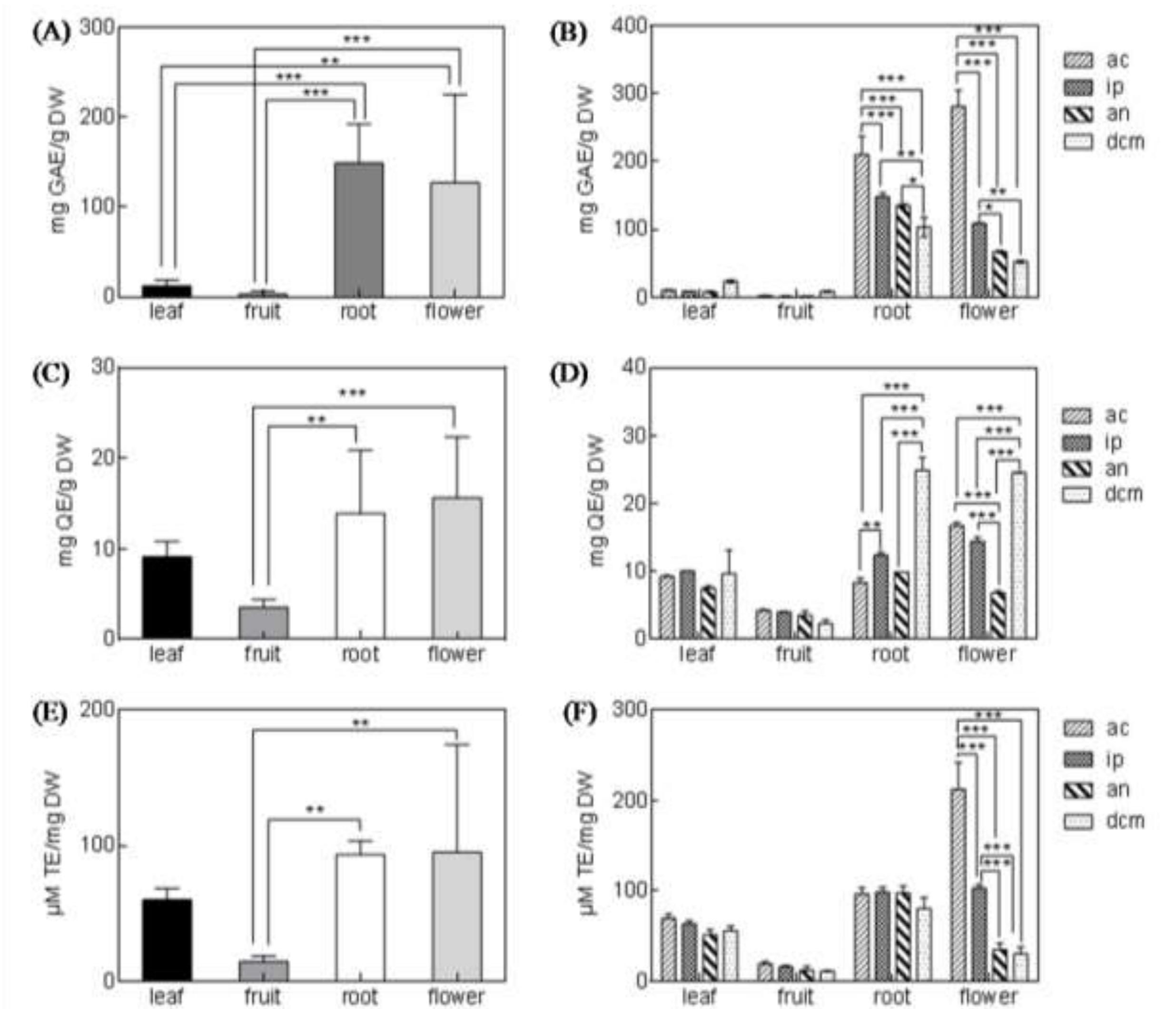

Figure 1:- TPC (A, B), TFC (C, D) and TAC (E, F) in *T. lampas* extracts among plant parts and among studied solvents. ac– acetone, ip– isopropanol, an– acetonitrile, dcm– dichloromethane, GAE –gallic acid equivalent, QE –qurecetin equivalent, TE –α-tocopherol equivalent.Values are expressed as the mean of measurements ± standard deviation (n=3). Analysis was performed using ANOVA followed by Tukey's's test at $p < 0.05$. *Significant $p < 0.1$, **Significant $p < 0.01$, *** Significant $p < 0.001$.

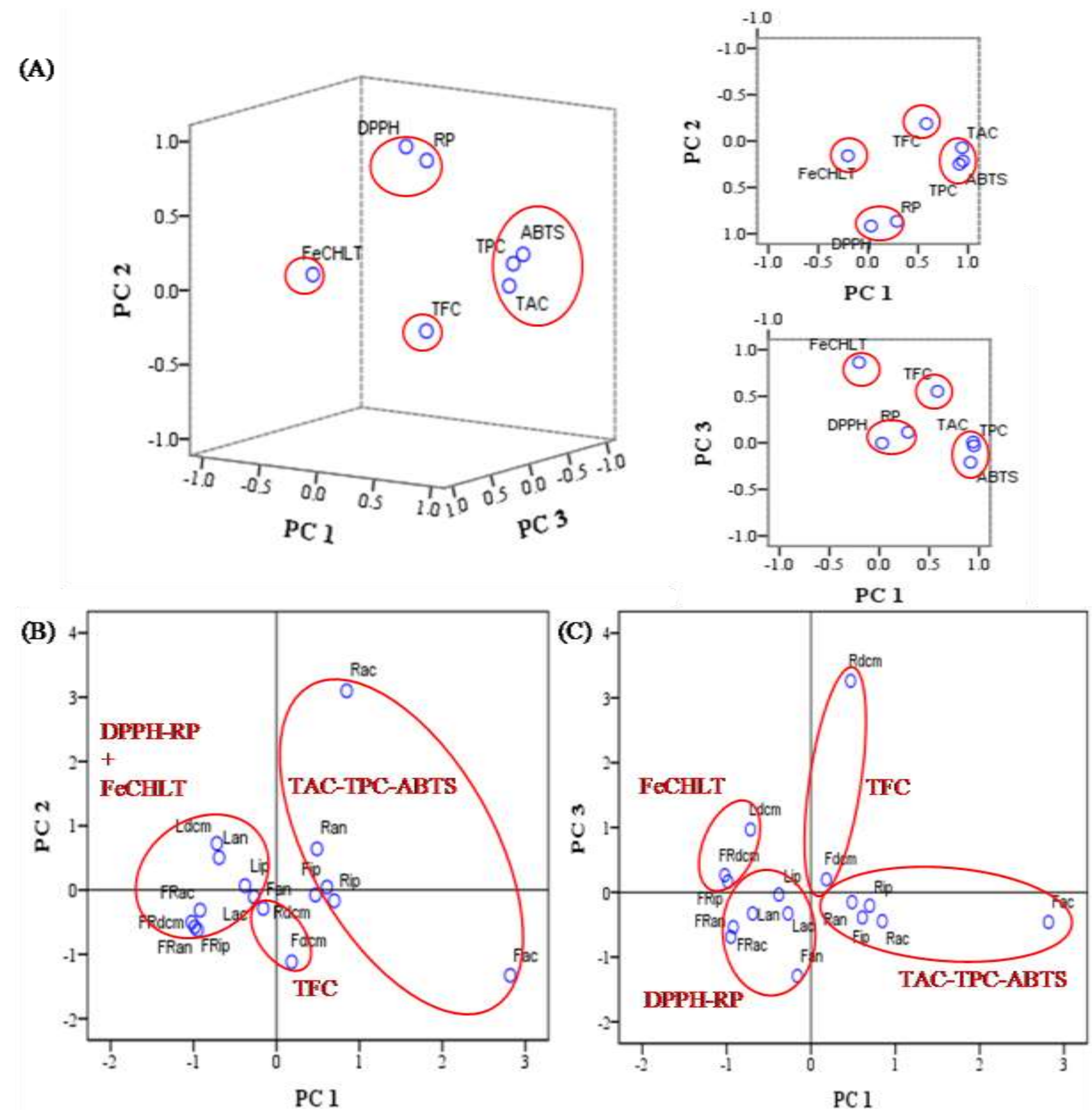

Figure 2:- Graphical representation of *T. lampas* extract in diverse solvent in the rotated space identified by PC1, PC 2 and PC 3 (A). Score plot between PC 1 and PC 2 (B) and score plot between PC 1 and PC 3 (C) based on principal component analysis of the extracts to their characteristic phytochemicals and antioxidant activities.

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