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Research Article

Phytochemical Studies of *Tephrosia purpurea* Linn. and *Martynia annua* Linn. extracts for Identification of Chemical Constituents

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ABSTRACT

Present study was aimed to study phytochemical study for qualitative screening of *Martynia annua* Linn. and *Tephrosia purpurea* Linn. for chemical constituents. Extraction of aerial parts of *T. purpurea* and *M. annua* leaves was performed by using ethyl alcohol, ethyl acetate, chloroform and petroleum ether. Flavonoid rich extracts of *M. annua* and *T. purpurea* was obtained from ethyl alcohol extract. Selected flavonoid rich fraction of both plant was further assess by thin layer chromatography for the pattern of chemical constituents. After development of solvent system both fractions was used for high performance thin layer chromatography (HPTLC). Results were confirmed that percent yield of fractions were calculated as 56.1%w/w (TP-A) and 4.2%w/w (MA-C). Both fraction was confirmed the presence of quercetin. In conclusion both plants and *T. purpurea* and *M. annua* extract contains quercetin as 66.35 mg and 28.42 mg, respectively determined by HPTLC method. These two plants are rich source of flavonoids that may be useful for treatment of various diseases.

Introduction

Tephrosia purpurea (Linn.) Pers. (Fabaceae) is a deep-rooted bush-like perennial herb, up to 60–90 cm high, remaining green throughout the dry season. It is highly branched suberect herbaceous perennial with thin firm glabrous branches leaves narrowly obtanceolate, green and glabrescent above, glaucous and obscurely silky beneath flower fascicled, pedicels short, bracteoks minute. Colour is brown with characteristic odour and aromatic taste. Tephrosin, deguelin, and quercetin are the most common chemical constituent of the aerial parts and roots of the plant.[1] The roots also contain isotephrosin and rotenone. Around 2.5% rutin has been found in roots and leaves. Rotenoids are formed in tissue cultures of plant components grown in vitro. Purpurin, a flavonone has been isolated from the seeds, as also 8-substituted flavonoid and 3-substituted oxygenated chalcones.[2] *T. purpurea* is used as tonic, laxative and deobstruent used in bronchitis and billious febrile attacks and also for boils, pimples and bleeding piles. Decoction of pods is used as vermifuge and to stop vomiting. Seeds oil said to be a specific against scabies, itch, eczema and other skin eruptions.[3] *T. purpurea* is traditionally used as digestive, antiulcer and antitussive in ayurvedic practice. It has been clinically tested in the treatment of viral hepatitis and is considered effective in various liver disorders.[4]

Martynia annua Linn. (Family Martyniaccae), is a glandular hairy annual that grows erect and branched. Leaves are opposite, roughly ovate to deltoid in shape, with a cordate base, sharp apex, and repand-dentate edges. Fruits are hard, woody with 2-sharp recurved hooks and seeds oblong.[1] The leaves contain chlorogenic acid and the seeds contain fatty acids (palmitic acid, stearic acid, and arachidic acid).[5] Gas chromatography–mass spectrometry (GC-MS) analysis of *M. annua* aqueous and alcoholic extracts revealed the presence of 28 chemicals,

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with oleic acid being the most abundant. Pelargonidin-3-5-diglucoside, cyanidin-3-galactoside, p-hydroxy benzoic acid, gentisic acid, arachidic acid, linoleic acid, palmitic acid, stearic acid, apigenin, apigenin-7-oglucuronide, p-hydroxy benzoic acid, gentisic acid, arachidic acid, linoleic acid, palmitic acid.[6]

M. annua is used to treat epilepsy and the tuberculosis gland in the neck. The fruit is useful in inflammation, scabies, painful urination. The juice of the leaves is used as a gargle for sore throats, the fruit for inflammation, and the leaf paste for dangerous insect stings and domestic animal wounds.[7] Aim of present study was to investigate phytochemical screening of *T. purpurea* and *M. annua* extracts to find out profile of different chemical constituents.

Material and Methods

Identification of Plant Material

The aerial part of *T. purpurea* Linn. was collected in the month of February to March from roadside of the campus of RKDF University Bhopal (M.P.). The leaves of *M. annua* Linn. was collected in September to October around the campus of RKDF University Bhopal (M.P.). *T. purpurea* Linn. and *M. annua* Linn. were identified and authenticated in the Department of Botany, Barkatullah University, Bhopal (M.P.). The plant materials were dried in shade, powdered moderately and pass through sieve No. 10.

Extraction and Preparation of Extracts

The powdered plant material (100 gm) of *T. purpurea* Linn. (aerial parts) and *M. annua* Linn. leaves (100 gm) were successively extracted in a soxhlet apparatus with petroleum ether (60–80°C), Chloroform, Ethyl acetate, ethyl alcohol (95%) and finally with Chloroform water (by maceration process). After each extraction test was performed to see whether the drug had been completely exhausted or not. The completion of extract was confirmed by evaporating a few drops of the extract on the watch glass and ensuring that no residue remained after evaporating the solvent. After ethanol extraction the marc obtained was dried and macerated with chloroform water for 24 hrs repeatedly two to three times. The liquid extracts were collected in a tare conical flask. The solvent removed by distillation method. The last traces of solvent being removed under vacuum. The extract obtained with each solvent was weighed to a constant weight and percentage w/w yield was calculated.[8-10]

Successive solvent extracts were obtained from 100 gm of dry powdered plant materials *T. purpurea* Linn. (aerial parts) and *M. annua* Linn. leaves. Petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts were subjected for qualitative analysis.

Qualitative Analysis of Extracts

All extracts were subject to various qualitative analysis to detect the presence of plant constituents e.g., Alkaloids, glycosides, saponins, carbohydrates, phytosterols, tannins and phenolic compounds (flavonoids), Proteins and free amino acids.[11]

Tests for Alkaloids

A small portion of extract was shake with about 5 mL of 1.5%v/v hydrochloric acid and filtered. The filtrate was tested with the alkaloid reagents:

- Mayer's reagent
- Dragendorff's reagent
- Wagner's reagent
- Saturated picric acid solution

Tests for Glycosides

(a) Keller-Killiani Test

Small portion of the respective extract was shake with 1ml glacial acetic acid containing trace of ferric chloride. 1-mL of concentrated sulfuric acid was added carefully by the sides of the test tube. A blue color in the acetic acid layer and red color at the junction of the two liquids was indicating presence of glycosides.

(b) Legal's Test

A little fraction of respective extract was take in water and made it alkaline. To alkaline solution few drops of sodium nitropruside solution were added. A blue color was indicating the presence of glycosides.

(c) Bontragger's Test

A small fraction of extract was dissolved in 1ml of benzene and then 0.5 mL of dilute solution of ammonia was added to the benzene solution. A rose pink to red color was indicating the presence of glycosides.

Tests for Saponins

(a) Little fraction of the extract was boiled with about 1-mL of distilled water with shaking. Appearance of a characteristic foam formation indicates the presence of saponins.

(b) A little fraction from extract was taken with about 2 mL of distilled water. Small quantity of sodium carbonate was added with shaking. The characteristic foam formation indicates the presence of saponins.

(c) Haemolysis Test: Small quantity of extract was diluted 5 times with normal saline. In series of 5 test tubes, doses of 0.2, 0.4, 0.6, and 0.8 mL were added and volume make in each test tube, up to 1-mL with normal saline. 10.5 ml of rabbit's blood was diluted to 25 mL with normal saline and 1-mL of the dilute blood was added to each test tube. A drop from each test tube was viewed under microscope confirms the haemolysis.

Tests for Phytosterols

(a) Hesse's reaction

With a few drops of chloroform, a small fraction of the extract was extracted, and an equivalent volume of

concentrated sulfuric acid was added to it with the test tube's sides. Appearance of a blood red color confirmed the presence of sterols in test sample.

(b) Liebermann's reaction

Warming a little quantity of the extract with around 1ml of acetic anhydride dissolved it. After cooling the contents, a few drops of strong sulphuric acid were applied to each test tube by the sides. Appearance of blue color indicated the presence of sterols.

(c) Liebermann's burchard reaction

In each example, a small fraction of the extract was combined with about 1-mL chloroform and a few drops of acetic anhydride, followed by about 0.5 mL concentrated sulphuric acid. The development of transient color was indicative of the presence of sterols in test sample.

Tests for carbohydrates

A solution of barium hydroxide was added to the extract until no fraction precipitate appeared. By passing CO_2, the excess barium was removed, and the resultant solution was filtered. The filtrate was neutralized with acetic acid.

The neutral solution was tested with molisch's reagent, fehling's solution, benedicts solution and tollen's reagents. The positive reaction was confirmed the test for presence of reducing sugars in sample.

(a) Molisch's test

A small fraction from extract was taken in ethanol separately and a few drops of 20%, w/v solution of α-napthol in ethanol added to it. After shaking well, about 1ml of concentrated sulphuric acid was allowed to flow carefully by the side of the test tube. A reddish violet ring at the junction of the two layers, indicate the presence of carbohydrates.

(b) Fehling's test

A small fraction from extract was taken with about 1ml of distilled water separately and filtered. Te filtrates were taken in test tubes separately and 1ml of Fehling's solution (A and B mixed together) was added to each test tube. The contents were placed in a boiling water bath for 2 minutes. Appearance of brick red precipitate of cuprous oxide indicated the presence of reducing sugars.

(c) Tollen's test

A little portion from extract was taken with a small portion of the distilled water and filtered. A few drops of the ammonical silver nitrate solution (Tollen's reagent) was added to filtrate and kept in boiling water both for 5 mins. Appearance of a silver mirror along the sides of the test tubes indicated the presence of reducing sugars.

(d) Barfoed reagent test

A little portion from extract was taken with 2 mL of distilled water and filtered. Then a small volume of Barfoed reagent was added to test tube and kept in a boiling water bath for 2 min. Appearance of red precipitate indicated the presence of monosaccharides.

Tests for Tannins and Phenolic Compounds

A small fraction of extract was dissolve in about 2 mL of distilled water and filter. The filtrate was tested with the following reagents.

(a) Ferric chloride solution

Appearance of blue to bluish green or bluish black color indicates presence of tannins and phenol compounds.

(b) Lead acetate solution

Appearance of brownish yellow precipitate indicates the presence of tannins.

(c) Potassium chromatic solution

Appearance of orange yellow precipitate indicates the presence of tannins.

Tests for Flavonoids

(a) Shinoda test

Plant extract was treated with magnesium and concentrated HCl, usually in ethanol solution. Addition of the acid drop wise to an alcohol solution containing few fragment of magnesium ribbon carried out the test. Characteristic color produced within a minute or two and the subsequent addition of more acid often causes modification of the color in a manner characteristic of the compound being examined. The test is positive with production of pink, scarlet, crimson red color for flavonoids.

(b) Ferric chloride solution

A small fraction of extract was taken in test tube and added 5% ferric chloride solution. Produces green purple or brown color in presence of flavonoids.

Tests for proteins and amino acids

(a) Millon's reaction

A small fraction of extract was taken in ethanol and filter. To about 2 mL of the filtrate, 5 to 6 drops of Millon's reagent was added; yellowish- red precipitate was indicates the presence of proteins.

(b) Xanthoproteic reaction

A small fraction of extract was taken in ethanol and few drops of concentrated solution of nitric acid was added. Appearance of yellow color was indicating presence of proteins.

A little fraction of extract was taken in ethanol and filtered. The filtrate was used as such and also after removing tannins by lead acetate method. Then spots were applied on chromatographic paper, the spots were dried and the paper was sprayed with Ninhydrin reagent.

The paper was dried and then heated in an oven at 80°C for 5 min. Appearance of violet colored spots indicates the presence of free amino acids.

Thin Layer Chromatography of Ethanol Extract of *T. purpurea* Linn. and *M. annua* Linn.

Thin layer chromatography was performed to find out the number of constituents present in the respective extract. Different solvent systems of varying polarity were tried. The best solvent system was selected which shows best resolution as well as large number of spots. The dried ethanol extract was dissolved in ethanol and applied on chromatographic plate having silica gel G as a stationary substance. The spots were observed in UV chamber (365 and 254 nm). Following solvent systems were selected that show best separation of constituents.

The migration distance of substances on thin layer chromatograms are generally fixed but some factors like thickness of the layer, chamber saturation, air humidity, separation efficacy of solvent mixtures etc. which are all difficult to reproduce can exert a marked influence on R_f value.

$$Rf = \frac{\text{Distance of centre of spot from starting point}}{\text{Distance of solvent front from starting point}}$$

Adsorbent: Silica gel-G; Detecting agent: UV 254 and 365 nm; Color of spots: Brown, red, green, yellow; R_f value: 0.05-0.95

Fractionation of Ethanol Extract of *T. purpurea*

The powdered plant materials of *T. purpurea* Linn. (aerial parts) was extracted with petroleum ether (60–80°C) in a soxhlet apparatus for three days up to complete defatting. The defatted dried powdered material was extracted for 3 to 4 days with 95% ethanol. The ethanol extract from the filtrate was concentrated. With the help of a separating funnel, semisolid extract was suspended in distilled water with proper shaking and extracted with ethyl acetate repeatedly up to complete extraction. After removing the solvent from the ethyl acetate fraction, a brown powdered product (A) was obtained, which tested positive for flavonoids. The aqueous layer of fraction (B) was discarded.

Fractionation of Ethanol Extract of *M. annua*

The powdered plant materials *M. annua* Linn. leaves were extracted in a soxhlet apparatus with petroleum ether (60–80°C) for defatting. The defatted dried powdered material was extracted with ethanol (95%) for 3 to 4 days. The filtrate ethanol extract was concentrated and completely dried. The dried ethanol extract treated with chloroform repeatedly to obtained chloroform soluble fraction (A) and chloroform insoluble fraction. The chloroform insoluble fraction was dissolved in methanol repeatedly to obtained methanol insoluble (B) and methanol soluble fraction (C). The methanol soluble fraction (C) has greater yield selected for further study.

Thin Layer Chromatography of Selected Fractions

Thin layer chromatography of *T. purpurea* Linn. and *M. annua* Linn. fractions was performed to develop profile of number of chemical components present in fractions.

On a pre-coated silica gel plate, thin layer chromatography (TLC) (0.2 mm, Merck 60 F 254, Germany) was used as the stationary phase. *T. purpurea* Linn. extract fraction (TP-A) and *M. annua* extract fraction (MA-C) were spotted on plate, and run in mobile phase, Toluene: chloroform: acetone (34:24:35). The extract fractions were spotted on TLC plate and run in mobile phase. After solvent run up to ¾ distance, plate was removed and observed in the UV 254 and 366 nm.

High-performance Thin Layer Chromatography (HPTLC) Analysis

The HPTLC fingerprinting of flavonoid rich fraction of both *T. purpurea* and *M. annua* was carried out on a pre-coated silica gel plate (0.2 mm, Merck 60 F- 254, Germany) as the stationary phase and Toluene: chloroform: acetone (34:24:35) as a mobile phase. The sample solution was prepared by dissolving dry flavonoid rich fraction in methanol (10 mg/mL). The samples (10 mL) and standard quercetin (Sigma Aldrich, USA) were spotted on pre-coated silica gel aluminum plate (10 cm x 10 cm) as bands (width 6 mm; distance between two bands, 15 mm) with a 100 mL Hamilton syringe by using Linomat-5 applicator. Whole HPTLC system CAMAG was operated by winCATS software (CAMAG Scientific Inc., USA). After run of solvent in 20 cm x10 cm twin glass chamber, the developed plate was dried and placed in the ultraviolet (UV) chamber at 254/365 nm. The plate was scanned under densitometer (CAMAG Scanner 3; lamp, D2 lamp) and chromatograms were recorded.

Results and Discussion

The plant material *T. purpurea* Linn. (aerial parts) and *M. annua* Linn. (leaves) were collected and identified. The powdered materials of both plants were successively extracted with petroleum ether, chloroform, ethyl acetate, ethyl alcohol and chloroform water. The percent yields of each extract were calculated (Table 1). The percent yields of all extracts of *T. purpurea* Linn. were found 4.5% w/w (petroleum ether), 1.6%w/w (chloroform), 3.1%w/w (ethyl acetate), 4.8%w/w (ethyl alcohol) and 2.2%w/w (chloroform water). The different extracts of *M. annua* Linn. were showed percent yield: 5.1% w/w (petroleum ether), 3.8%w/w (chloroform), 3.4%w/w (ethyl acetate), 4.1%w/w (ethyl alcohol) and 2.3%w/w (chloroform water).

The phytochemical analyses of all extracts were performed qualitatively for different phytoconstituents. The plant *T. purpurea* Linn. give positive test of steroids in petroleum ether and alkaloids in chloroform extract. The ethyl acetate and ethanol extract contains flavonoids, tannins, glycosides and amino acids. Aqueous extract were

Table 1: Percentage yield of various extracts of *T. purpurea* Linn. and *M. annua* Linn.

Plant name	*Solvents used for extraction*	*Time required for complete extraction (hrs)*	*% yield (w/w)*
T. purpurea Linn.	Petroleum ether (60–80°C)	9	4.5
	Chloroform	12	1.6
	Ethyl acetate	11	3.1
	Ethyl alcohol (95%)	13	4.8
	Chloroform water	12	2.2
M. annua Linn.	Petroleum ether (60–80°C)	10	5.1
	Chloroform	10	3.8
	Ethyl acetate	12	3.4
	Ethyl alcohol (95%)	13	4.1
	Chloroform water	10	2.3

found presence of carbohydrates, saponins and amino acids. In case of *M. annua* Linn. petroleum ether extract contains steroids and chloroform extract were found presence of alkaloids. The ethyl acetate and ethanol extract contains flavonoids, tannins and glycosides. Aqueous extract contains carbohydrates, saponins and proteins (Tables 2 and 3).

The TLC of ethanol extracts of *T. purpurea* Linn. and *M. annua* Linn. were done in different solvent systems and spots as well as best separation was observed (Tables 4 and 5).

Based on phytochemical analysis and previous study we had selected ethanol extract of *T. purpurea* Linn. and *M. annua* Linn. for detailed study. For the detail phytochemical study, further extraction of both plant materials was done with ethanol in large quantity. The two kg plant material of both plants was taken for extraction with ethanol and

Table 2: Qualitative analysis of different extracts of *Tephrosia purpurea* Linn.

Tests	*Pet. Ether*	*Chloroform*	*Ethyl acetate*	*Alcohol*	*Aqueous*
Test for sterols					
Liebermann's reaction	+	+	-	-	-
Liebermann-Burchard's test	+	+	-	-	-
Hesse's reaction	+	+	-	-	-
Test for Glycoside					
Bontrager	-	-	+	+	+
Kellar-killiani test	-	-	+	+	-
Legal's test	-	-	+	+	+
Test for saponins					
Foam test	-	-	-	+	+
Haemolysis test	-	-	-	+	+
Test for carbohydrates					
Molisch's test	-	-	-	+	+
Barford's test	-	-	-	+	+
Fehling test	-	-	-	+	+
Tollen's test	-	-	-	+	+
Test for alkaloids					
Mayer's test	-	-	-	-	-
Wagner's test	-	-	-	-	-
Dragendorff's test	-	-	-	-	-
Hagger's test	-	+	-	-	-
Test for Flavanoids					
Ferric chloride	-	-	+	+	+
Shinoda test	-	-	+	+	+
Test for Tannins					
Ferric chloride	-	-	+	+	+
Gelatin test	-	-	+	+	+
Test for Protein and amino acid					
Millon's test	-	-	+	+	+
Xanthoproteic test	-	-	+	+	+
Ninhydrin test	-	-	-	+	+

- Negative, + Positive

Table 3: Qualitative analysis of different extracts of *Martynia annua* Linn.

Tests	*Pet. Ether*	*Chloroform*	*Ethyl acetate*	*Alcohol*	*Aqueous*
Test for sterols					
Liebermann's reaction	+	+	-	-	-
Liebermann - Burchard's test	+	+	-	-	-
Hesse's reaction	+	-	-	-	-
Test for glycoside					
Bontrager	-	-	+	+	+
Kellar-killiani test	-	-	-	+	-
Legal's test	-	-	-	+	-
Test for saponins					
Foam test	-	-	-	+	+
Haemolysis test	-	-	-	+	+
Test for carbohydrates					
Molisch's test	-	-	-	+	+
Barford's test	-	-	-	+	+
Fehling test	-	-	-	-	+
Tollen's test	-	-	-	-	+
Test for alkaloids					
Mayer's test	-	+	-	+	-
Wagner's test	-	+	-	+	-
Dragendorff's test	-	+	-	+	-
Hagger's test	-	-	-	-	-
Test for flavanoids					
Ferric chloride	-	-	+	+	+
Shinoda test	-	-	+	+	+
Test for Tannins					
Ferric chloride	-	-	+	+	+
Gelatin test	-	-	+	+	+
Test for protein and amino acid					
Millon's test	-	-	-	+	+
Xanthoproteic test	-	-	-	+	+
Ninhydrin test	-	-	-	+	+

collect dry ethanol extract. Fraction of ethanol extract was carried out with different organic solvents. From ethanol extract of *T. purpurea* Linn. two fractions (TP-A and TP-B) and three fractions (MA-A, MA-B and MA-C) from ethanol extract of *M. annua* Linn. were obtained. The percent yield of fractions were calculated as 56.1%w/w (TP-A), 3.8%w/w (TP-B), 3.8%w/w (MA-A), 2.1%w/w (MA-B) and 4.2%w/w (MA-C). The results are shown in Table 6.

Thin layer chromatography of all fractions was carried out with different solvent systems. The TLC of *T. purpurea* Linn. fractions (TP-A and TP-B) was observed better separation in benzene: chloroform: methanol (12:10:02) solvent system. The good resolution in spots for *M. annua* Linn. fractions (MA-A, MA-B and MA-C) was observed in

Table 4: Different solvent systems for TLC of ethanol extract of *T. purpurea* Linn.

S.N.	*Solvent systems*	*No. of spots*
1.	Benzene : Chloroform (50:50)	2
2.	Benzene : Chloroform : Methanol (40:10:1)	3
3.	Benzene : Chloroform : Methanol (20:5:1)	3
4.	Benzene : Chloroform : Methanol (15:6:2)	3
5.	Benzene : Chloroform : Methanol (14:9:1)	5
6.	Benzene : Chloroform : Methanol (12:9:1)	6
7.	Benzene : Chloroform : Methanol (12:9:1.5)	8
8.	Benzene : Chloroform : Methanol (12:10:2)	9
9.	Toluene : Chloroform: Acetone (45:20:32)	9

Table 5: Different solvent systems for TLC of ethanol extract of *Martynia annua* Linn.

S.N.	*Solvent systems*	*No. of spots*
1.	Toluene: Ethyl acetate (10:2)	4
2.	Ethyl acetate: n-butanol: Formic acid: Water (12:9:2:1)	6
3.	Benzene: Ethylacetate (4:3)	7
4.	Benzene : Chloroform : Methanol (12:10:2)	8
5.	Benzene : Chloroform : Methanol (25: 35: 10)	8
6.	Toluene: chloroform: Acetone (32:22:40)	7
7.	Toluene: chloroform: Acetone (34:24:35)	8

Table 6: Percent yield of *T. purpurea* Linn. and *M. annua* Linn. fractions

Name of plant	*Fractions*	*% (w/w) yield*
T. purpurea Linn.	TP-A	6.1
	TP-B	3.8
M. annua Linn.	MA-A	3.8
	MA-B	2.1
	MA-C	4.2

TP- *T. purpurea* Linn.; MA- *M. annua* Linn.

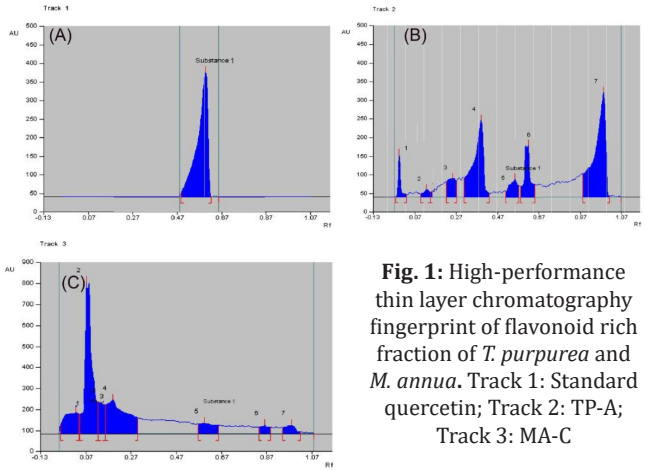

Fig. 1: High-performance thin layer chromatography fingerprint of flavonoid rich fraction of *T. purpurea* and *M. annua*. Track 1: Standard quercetin; Track 2: TP-A; Track 3: MA-C

toluene: chloroform: acetone (34:24:35) solvent system. The spots were observed in UV 365 nm.

The HPTLC fingerprinting of flavonoid rich fraction was revealed several peaks under UV 365 nm (Fig. 1). The standard compound quercetin gave single major spot at Rf value 0.54. The TP-A gives seven clear peaks with the range of Rf values 0.0-0.98. The compound with Rf value 0.54 is identify as querctin. The densitometry scan was performed for all tracks at 365 nm and identity the presence of quercetin in flavonoid rich fraction of both plants. The amount of quercetin as per 100 gram of extract was 66.35 mg and 28.42 mg for *T. purpurea* and *M. annua* extract, respectively.

Conclusion

Flavonoids are potent health-promoting substances as they have act as antioxidant and anti-inflammatory properties. Potent antioxidant and anti-inflammatory agents such as quercetin and luteolin can play an important role in restoring physiological conditions, allowing a significant improvement in healing. Results of present study was concluded that *T. purpurea* and *M. annua* extract contains quercetin as 66.35 and 28.42 mg, respectively determined by HPTLC method. These two plants are rich source of flavonoids that may be useful for treatment of various diseases.

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