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Phytochemical analysis and estimation of phytoconstituents in coded plant (222) leaves – Part VII

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Abstract

In the present study, the preliminary phytochemical analysis and the estimation of phytochemical constituents were carried out in coded plant 222 leaves. Cold extraction method was used for the preparation of the leaf extract using ethanol as solvent. Phytochemical screening of the coded plant 222 leaf extract showed the presence of carbohydrates, steroids, phenolics and flavonoid compounds. The quantitative phytochemical analysis was carried out to estimate the total phenolic, flavonoid and condensed tannin contents. Total phenolic content, flavonoid and condensed tannin contents of coded drug 222 ethanolic extract were estimated as 107.00 mg/g Gallic Acid Equivalents, 132.54 mg/g Quercetin Equivalents and 187.90 mg/g Catechin Equivalents respectively. Chemical profiling of the extract was also carried out by Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) methods. Results of the present study suggested that the extract contains considerable amount of phenolic compounds and flavonoids and the presence of such phytocompounds is responsible for the therapeutic effects exhibited by the coded plant 222 leaves.

Keywords: TLC, HPTLC, Phenolic compounds, Flavonoids

1. Introduction

Human beings used medicinal plants for various purposes from the beginning of the human history. Traditional folk remedies from medicinal plants have always guided researchers to search for new medications in order to maintain and promote healthy life. It played a crucial role in the process of drug discovery (Farnsworth, 2008). Herbal medicines have recently attracted much attention as alternative medicines for the treatment or preventing life style related diseases and relatively very little knowledge is available about their mechanism of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Plants are the essential and integral part in complementary and alternative medicine. They are the best sources of active secondary metabolites which are beneficial to mankind in treating many diseases.

Phytochemical screening is a valuable step in the detection of the bioactive compounds present in medicinal plants and subsequently may lead to drug discovery and development. Medicinal plants have bioactive compounds which are used for curing of various human diseases.

The present phytochemical study was carried out based on the traditional claim related to a coded medicinal plant (Code No. 222) disclosed by a traditional healer from Karunagappally, Kollam

district, Kerala and the claim was that he was using the particular medicinal plant species against diabetes and related complications and it also provides hepatoprotection. We have reported the hepatoprotective activity of coded plant 222 leaf ethanolic extract against various hepatotoxicants such as paracetamol, carbon tetrachloride, alcohol and D-Galactosamine induced liver damages (Krishnakumar *et al*., 2017 a, b; Krishnakumar *et al*., 2018 a, b). We have already reported the anti-diabetic effects of the coded plant 222 leaf ethanolic extract in alloxan and streptozotocin induced diabetic models (Krishnakumar *et al*., 2016 a, b). On verification, no phytochemical studies related to the coded plant 222 leaves had been so far conducted. The phytochemical analysis and the quantitative estimation of phytochemical constituents in coded plant 222 leaves were carried out in the present study.

2. Materials and Methods

2.1. Preparation of the plant leaf extract

The coded plant 222 leaves were collected from the Traditional Healer at Karunagappally, Kollam District, Kerala. They were authenticated by Dr. Mathew Dan, Plant taxonomist of the Institute and a voucher specimen (TBGT 57057 dated 16/10/2009) was deposited at the Institute Herbarium. The leaves were washed thoroughly, shade dried, powdered and 100 g of the leaf powder was extracted with 1000 mL of ethanol overnight, at room temperature with constant stirring (200 rpm). The extract was filtered, concentrated and the solvent evaporated completely in a rotary evaporator at 40°C under reduced pressure, dried in a desiccator and used for the experiments.

2.2. Chemicals

Sodium carbonate, Gallic acid, Aluminium chloride, Potassium acetate, Quercetin and Catechin were purchased from Sigma Aldrich, USA. Folin-Ciocalteu reagent and Vanillin reagent were purchased from Hi Media, Mumbai, India.

2.3. Preliminary phytochemical analysis

The preliminary phytochemical analysis of the crude extract of coded drug 222 was carried out according to the method of Harborne (1984).

2.3.1. Alkaloids

Mayer's Test: One or two drops of Mayer's reagent [$HgCl_2$ (1.36g), dissolved in 60 ml distilled water and mixed with a solution of 5 g of KI in 10 ml water] was added to the acidified plant extract. The formation of a white precipitate indicated the presence of alkaloids.

2.3.2. Flavonoids

(a). Shinoda's Test: A few milligrams of the extract was dissolved in a few ml of methanol and magnesium turnings was added followed by 5 ml HCl. Flavonoids give a pink colour.

(b). Alkaline Reagent Test: To 2 ml of the extract, few drops of NaOH was added. At first intense yellow colour was formed, which was subsequently turned to colourless on addition of a few drops of dilute acid.

2.3.3. Terpenoids/Steroids

Liebermann-Burchard Reaction: Freshly prepared LB reagent (5ml acetic anhydride and 5 ml sulphuric acid) was added to the extract. Presence of steroids and terpenoids was indicated by the presence of green colour at the top and pink colour at the bottom of the test tube respectively.

2.3.4. Saponins

Formation of froth which lasts for long time when the sample was mixed with water and shaken well was taken as indication of saponins.

2.3.5. Coumarins

A little of the extract was dissolved in methanol and alcoholic KOH or NaOH was added to the solution. A yellow colour which disappeared on adding conc. HCl indicated the presence of coumarins.

2.3.6. Tannins

Ferric Chloride Test: To few ml of extract add $FeCl_3$ (10%) solution. The formation of green colour indicates the presence of condensed tannnins.

2.3.7. Glycosides

(a). Borntrager's Test: The test extract is boiled with 1 ml of conc. H_2SO_4 for 5 minutes. It is filtered, and the filtrate is then treated with 10% ammonium solution. Pink colour indicates the presence of glycosides.

(b). Legal's Test: 50 mg of the extract is dissolved in pyridine sodium nitroprusside solution and is

made alkaline using 10% NaOH. Presence of glycoside is indicated by pink colour.

2.3.8. Phytosterols

Liebermann Burchard Test: To the chloroform solution of extracts, added acetic anhydride and conc. H_2SO_4 from the sides of the test tubes and allowed to stand. The brown ring was formed at the junction of two layers. The upper layer acquired green colour showing the presence of phytosterols.

2.3.9. Proteins

Ninhydrin test: Few drops of ninhydrin were added to the extract. Appearance of blue colour indicates the presence of amino acids whereas proteins may rarely give positive result.

2.4. Chemical profiling

Chemical profiling is usually tested by TLC and HPTLC. The characteristic fingerprint profile of thin layer chromatograms can be used as marker for the quality evaluation of a particular sample.

2.4.1. Thin Layer Chromatography (TLC)

(a) Test solution: 2 g of powdered drug is extracted with methanol (20 ml) using a magnetic stirrer by continuous shaking (4 h) at room temperature and filtered. The filtrate is concentrated under reduced pressure. Make the concentration of the extract dissolved in methanol to 10 mg/ml.

(b) Solvent system: Hexane: Chloroform: Methanol (5:4:1).

(c) Procedure: 5 µL of the test solution applied on precoated silica gel plate (E. Merck) of uniform thickness of 0.2 mm with the help of a sample spotter. The plates are developed in the solvent system to a distance of 8 cm. The plates were sprayed with anisaldehyde reagent and heated at 105^0C for 5 min.

A number of bands are visible when anisaldehyde reagent was sprayed both in UV 254 nm and UV 366nm.

2.4.2. High Performance Thin Layer Chromatography (HPTLC)

(a) Test solution: 2 g of powdered drug extracted with methanol (20 ml) on a magnetic stirrer by continuous shaking (4 h) filtered and the filtrate concentrated under reduced pressure. The extract is dissolved in methanol to make a solution of 10 mg/ml concentration.

(b) Solvent system: Hexane: Chloroform: Methanol (5:4:1).

(c) Procedure: The extracts were estimated using a HPTLC system (CAMAG, Switzerland) made up of a Linomat V sample applicator, a CAMAG twin-trough plate development chamber, CAMAG TLC scanner 3 and WinCATS software 4.03.

10 µl of the test solution applied on to silica gel HPTLC plates (60 F-254, E. Merck, Germany) as 6 mm wide bands with the automatic Linomat V sample applicator.

2.5. Estimation of phytoconstituents

The phytoconstituents such as total phenols, total flavonoid and condensed tannin content were estimated by standard methods.

2.5.1. Estimation of total phenolic content

The total phenolic content in the plant extracts were determined using Folin-Ciocalteu method (Singleton and Rossi, 1965). To 1 ml of standard solution at different concentrations, added 5 ml Folin-Ciocalteu reagent (1:10 dilution in distilled water). 4 ml of 7.5 % sodium carbonate was added to all the samples after 8 min. The solution was incubated for 2 h at room temperature and the absorbance was measured at 765 nm using UV/VIS spectrophotometer (Agilent Technologies, USA). 1 ml of (1 mg/ml) plant extract was taken as test sample and the process repeated. A standard curve was prepared using gallic acid at different concentrations prepared in ethanol. The results were expressed as mg gallic acid equivalents per g dry weight (mg GAE/g DW).

2.5.2. Estimation of total flavonoids

Aluminium chloride colourimetric method was used for total flavonoid determination (Kale *et al.,* 2010). The plant extracts (0.5 ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm by spectrophotometric method. The calibration curve was plotted from standard quercetin at different concentrations. The results were expressed as mg quercetin equivalents per g dry weight (mg QE/g DW).

2.5.3. Estimation of condensed tannins

Condensed tannin or proanthocyanidins were estimated according to vanillin-HCl method

(Makkar and Becker, 1993). One part of 1% Vanillin reagent was mixed with one part of 8% HCl solution just before use. The test extracts were prepared in 1 mg/ml concentration. 0.1 to 1.0 ml catechin standard was taken in a series of test tubes and made up to 1 ml with methanol and added 5 ml Vanillin reagent to each tube at an interval of 1 min. The reaction mixture was incubated for 20 min. at room temperature and the absorbance was taken at 500 nm. The results were expressed as mg catechin equivalents per g dry weight (mg CE/g DW).

2.6. Statistical analysis

All the analyses were carried out in triplicate. The statistical significance between the samples were determined by the Analysis of Variance (ANOVA) and the data were recorded as mean ± Standard Deviation (SD). $P \leq 0.05$ was considered to be statistically significant. Significant differences between means were determined by Duncan's Multiple Range tests (Raghava, 1987). The computer software employed for the statistical analysis was IBM SPSS Statistics, version 20 (USA).

3. Results and Discussion

The therapeutic effects of medicinal plants are mainly due to the presence of various secondary metabolites such as flavonoids, terpenoids, alkaloids, glycosides, phenols, sterols etc.

The preliminary phytochemical analysis is useful in the detection and isolation of bioactive principles and may subsequently lead to drug development. In the present study, the preliminary phytochemical analysis of the ethanolic leaf extract of coded drug 222 revealed the presence of important antioxidant phytochemicals such as phenolic compounds. The crude extract of coded drug 222 is rich in flavonoids and steroids, which may be responsible for its therapeutic effects (Table 1).

Different solvent combinations were tried after continuous trials to develop maximum separation of bands in the TLC system. The most suitable solvent system for the leaf ethanolic extract of coded drug 222 was found to be hexane: chloroform: methanol in the ratio 5:4:1, in which maximum separation of the phytocompounds was visualised (Fig. 1). Table 2 represents TLC profile of the extract with R_f values and band colour. HPTLC fingerprint is an important quality assessment tool for the evaluation of plant extracts because, a large number of compounds can be analysed efficiently and cost effectively.

Table 1. Results of the preliminary phytochemical analysis of the coded drug 222 leaf ethanolic extract

Sl. No.	Phytoconstituents	Present (+ ve) / Absent (- ve)
1	Carbohydrates	+ ve
2	Saponins	- ve
3	Steroids	+ve
4	Coumarins	-ve
5	Flavonoids	+ve
6	Alkaloids	-ve
7	Terpenoids	-ve
8	Phenolic compounds	+ ve

This technique allows the assessment of numerous samples in a single analysis. The sameanalysis can be viewed collectively in different wavelengths of light producing complete profile of the plant extract (Kunle *et al.*, 2012). The solvent system which gives maximum separation of compounds in TLC was used as the solvent system for HPTLC analysis (hexane: chloroform: methanol in the ratio 5:4:1). The plates were scanned at different wavelenghths and they were derivatised using anisaldehyde sulphuric acid spray reagent, heated at 110^0C for 10 min. and observed. The presence of multiple peaks in the densitometric scan indicated the presence of different compounds in the extract. Fig. 2-4 represent the HPTLC profile of the leaf ethanolic extract of coded drug 222 and the corresponding peak data are shown in Table 3 and Table 4 respectively.

Table 2. TLC profile of ethanolic extract of leaves coded drug 222

R_f value	Colour of the band
0.90	Light pink
0.80	Light pink
0.72	Pink
0.61	Light green
0.56	Pink
0.50	Light pink
0.25	Grey
0.18	Grey
0.16	Grey
0.13	Dark grey
0.01	Dark grey

Table 3. HPTLC peak table of ethanolic extract of Coded drug 222 leaves scanned at 366 nm

Peak	Start position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %
1	0.01 R_f	32.1 AU	0.02 R_f	91.4 AU	4.99 %	0.08 R_f	40.7 AU	3963.9 AU	5.75 %
2	0.08 R_f	40.9 AU	0.12 R_f	83.2 AU	4.54 %	0.15 R_f	38.8 AU	3059.4 AU	4.43 %
3	0.15 R_f	39.0 AU	0.20 R_f	207.0 AU	11.31 %	0.22 R_f	79.7 AU	4843.2 AU	7.02 %
4	0.22 R_f	80.4 AU	0.23 R_f	112.9 AU	6.16 %	0.25 R_f	61.9 AU	2362.5 AU	3.42 %
5	0.25 R_f	61.9 AU	0.30 R_f	280.5 AU	15.32 %	0.33 R_f	68.2 AU	9565.9 AU	13.87 %
6	0.33 R_f	68.4 AU	0.34 R_f	70.4 AU	3.84 %	0.39 R_f	47.9 AU	2758.2 AU	4.00 %
7	0.39 R_f	48.0 AU	0.43 R_f	59.6 AU	3.26 %	0.47 R_f	21.4 AU	2940.3 AU	4.26 %
8	0.47 R_f	21.7 AU	0.53 R_f	46.7 AU	2.55 %	0.55 R_f	42.5 AU	2979.0 AU	4.32 %
9	0.56 R_f	42.5 AU	0.60 R_f	69.2 AU	3.78 %	0.64 R_f	38.1 AU	3840.8 AU	5.57 %
10	0.64 R_f	38.2 AU	0.69 R_f	57.3 AU	3.13 %	0.69 R_f	56.8 AU	2058.3 AU	2.98 %
11	0.70 R_f	57.1 AU	0.78 R_f	298.9 AU	16.33 %	0.81 R_f	40.0 AU	14418.0 AU	20.90 %
12	0.81 R_f	140.0 AU	0.84 R_f	453.6 AU	24.78 %	0.96 R_f	1.5 AU	16195.2 AU	23.48 %

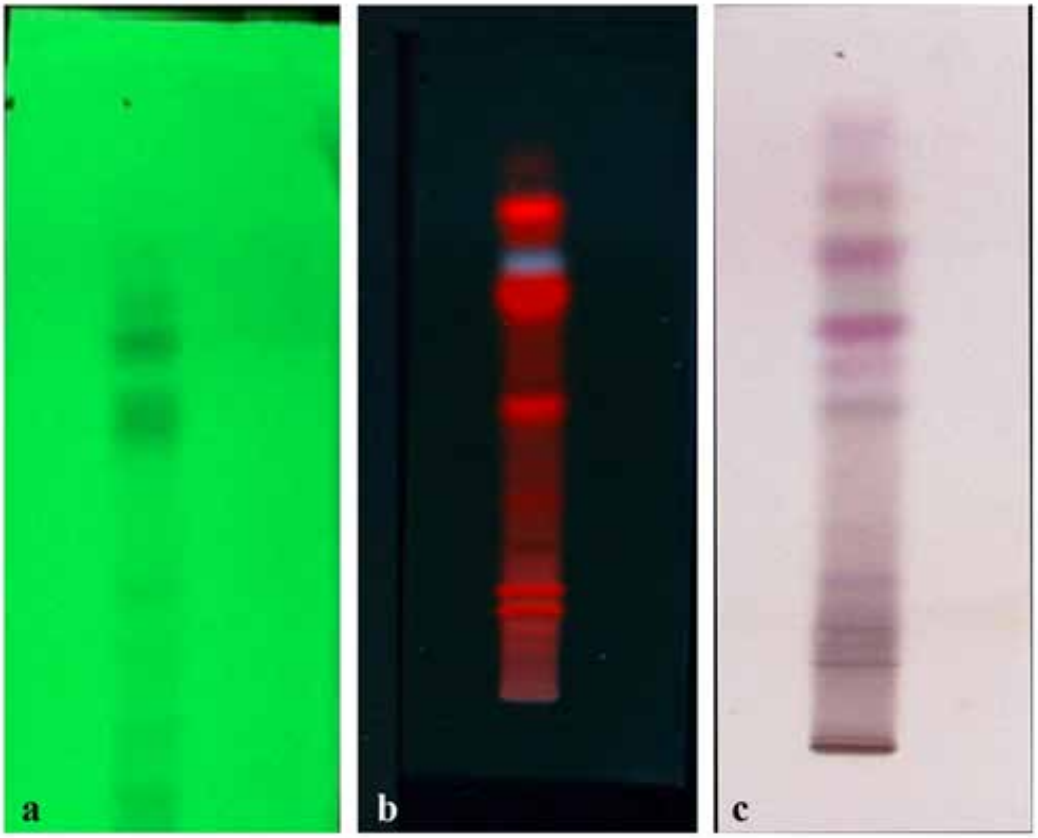


Fig. 1. TLC profile of the ethanolic leaf extract of Coded plant powder 222; (a) Plate developed under UV-254 nm; (b) Plate developed under UV-366 nm; (c) Derivatised plate under white light

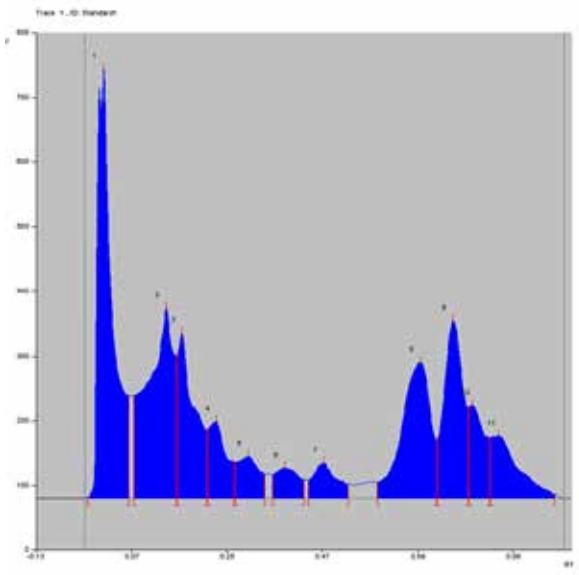


Fig. 2. HPTLC profile of the ethanolic leaf extract of Coded plant powder 222 – Graph peak display of the extract scanned at 254 nm

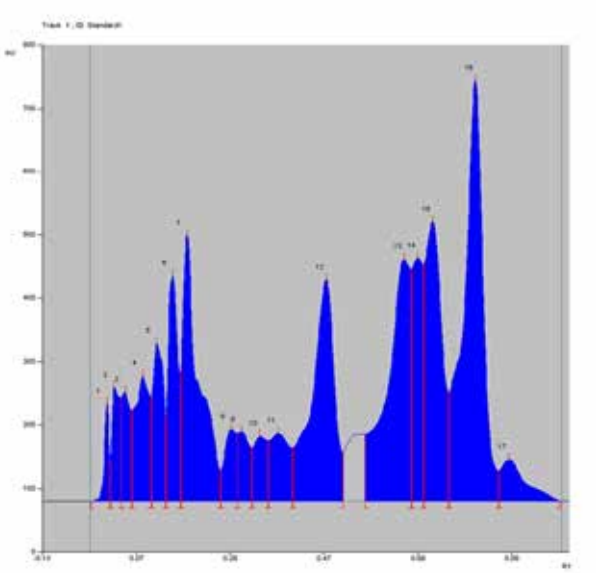


Fig. 3. HPTLC profile of the ethanolic leaf extract of Coded plant powder 222 – Graph peak display of the derivatised plate scanned at 366 nm

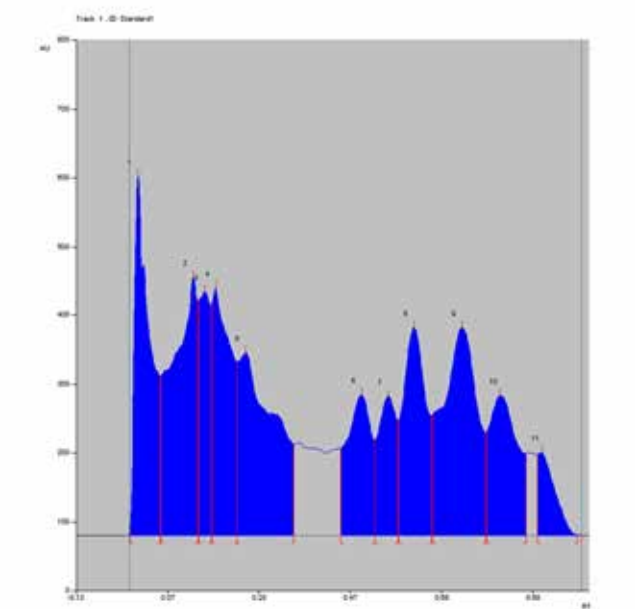
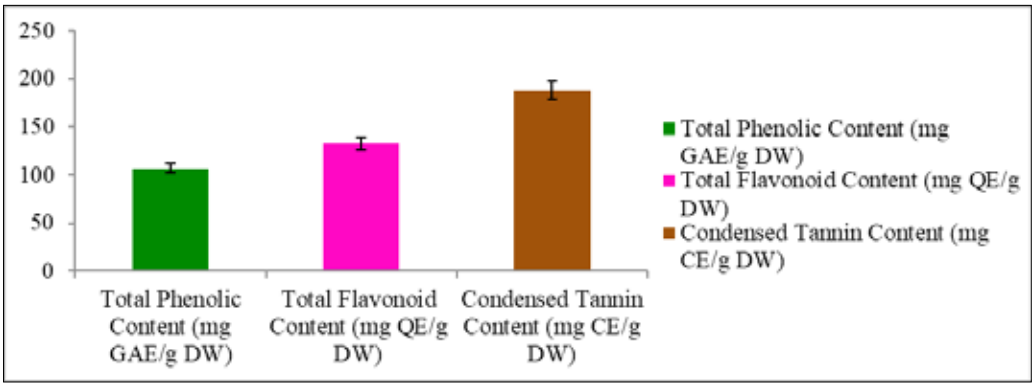


Fig. 4. HPTLC profile of the ethanolic leaf extract of Coded plant powder 222 – Graph peak display of the extract scanned after derivatisation at 580 nm

Table 4. HPTLC peak table of ethanolic extract of Coded drug 222 leaves scanned at 580 nm

Peak	Start position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %
1	0.00 R_f	16.2 AU	0.01 R_f	422.2 AU	19.69 %	0.03 R_f	38.7 AU	6928.7 AU	12.88 %
2	0.03 R_f	239.5 AU	0.04 R_f	272.4 AU	12.71 %	0.06 R_f	69.9 AU	5486.1 AU	10.20 %
3	0.06 R_f	173.2 AU	0.08 R_f	318.8 AU	14.87 %	0.14 R_f	10.8 AU	9398.9 AU	17.47 %
4	0.14 R_f	11.0 AU	0.16 R_f	30.5 AU	1.42 %	0.17 R_f	26.6 AU	484.9 AU	0.90 %
5	0.20 R_f	31.9 AU	0.22 R_f	152.9 AU	7.13 %	0.23 R_f	57.1 AU	2779.8 AU	5.17 %
6	0.24 R_f	57.7 AU	0.27 R_f	111.1 AU	5.18 %	0.29 R_f	94.3 AU	3773.6 AU	7.02 %
7	0.29 R_f	94.9 AU	0.30 R_f	120.1 AU	5.60 %	0.32 R_f	95.9 AU	2812.9 AU	5.23 %
8	0.33 R_f	98.2 AU	0.34 R_f	117.4 AU	5.48 %	0.36 R_f	82.9 AU	3088.8 AU	5.74 %
9	0.40 R_f	87.7 AU	0.40 R_f	95.7 AU	4.46 %	0.43 R_f	89.4 AU	2506.7 AU	4.66 %
10	0.43 R_f	69.6 AU	0.46 R_f	106.8 AU	4.98 %	0.55 R_f	19.6 AU	5887.0 AU	10.95 %
11	0.55 R_f	19.8 AU	0.55 R_f	21.2 AU	0.99 %	0.58 R_f	2.3 AU	353.4 AU	0.66 %
12	0.58 R_f	2.6 AU	0.63 R_f	86.5 AU	4.04 %	0.64 R_f	77.6 AU	2365.1 AU	4.40 %
13	0.64 R_f	78.7 AU	0.66 R_f	89.9 AU	4.19 %	0.67 R_f	62.6 AU	2319.9 AU	4.31 %
14	0.69 R_f	60.8 AU	0.70 R_f	78.7 AU	3.67 %	0.74 R_f	0.3 AU	2130.6 AU	3.96 %
15	0.74 R_f	0.9 AU	0.77 R_f	80.3 AU	3.75 %	0.81 R_f	8.1 AU	2257.3 AU	4.20 %
16	0.81 R_f	8.6 AU	0.84 R_f	39.1 AU	1.82 %	0.87 R_f	1.6 AU	1213.3 AU	2.26 %



Values are the mean ± SD, n=6 in each group, analysis of variance (ANOVA) followed by Duncan's multiple range test.

Fig. 5. Total phenolic content (mg GAE/g DW), total flavonoid content (mg QE/g DW) and condensed tannin content (mg CE/g DW) in the ethanolic extract of Coded drug 222 leaves

In the present study, the total phenolic content, total flavonoids and condensed tannin content of the ethanolic extract of coded drug 222 leaves were estimated. The phenolic content in the extracts were expressed as milligram gallic acid equivalents per gram dry weight (mg GAE/g DW). The total phenolic content in the ethanolic extract of coded drug 222 leaves was estimated as 107.00 mg GAE /g DW (Fig. 5). The presence of phenolic compounds indicated that the plant is an antimicrobial agent. Natural phenolic compounds exhibited diverse therapeutic effects such as antimicrobial (Ofokansi *et al*., 2005), antioxidant, immunomodulatory, anti-diabetic and anticancer. It also reduced the incidence of diseases including cardio-vascular diseases, osteoporosis and neurodegenerative diseases (Oliver *et al*., 2016).

The total flavonoid content was estimated by aluminium chloride colourimetric method. It was calculated from the standard graph of quercetin and expressed as quercetin equivalents in mg quercetin/g dry weight of different solvent extracts. The total flavonoid content in the leaf ethanolic extract of coded drug 222 was estimated as 132.54 mg QE/g DW (Fig. 5). Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage (Salah *et al*., 1995).

Condensed tannin or proanthocyanidins were estimated according to vanillin-HCl method and the results were expressed as mg catechin equivalents per g dry weight (mg CE/g DW). The estimation of condensed tannin from the ethanolic extract of coded drug 222 leaves was estimated as 187.90 mg CE/g DW (Fig. 5). Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membranes.

The quantification of total phenolics, flavonoids and other antioxidant constituents is very important because of their free radical neutralizing ability. Phenolic compounds are the most wide spread natural compounds present in plants. They play an important role in the mechanism of free radical scavenging by acting as hydrogen donators or metal chelators or singlet oxygen quenchers. These effects are due to the presence of multiple hydroxyl groups present in phenolic compounds (Nagulendran *et al*., 2007). Prevention of the free radicals formed by the metabolic processes is essential to maintain a healthy biological system. The scavenging of oxygen derived free radicals is an important property of flavonoids and thus they can act as natural antioxidants. The antioxidant mechanism of plant flavonoids is by direct scavenging of free radicals or by chelating process. The polyphenolic compounds and flavonoids are abundantly present in food and medicinal plants as glycosides and they contain many phenolic hydroxyl groups. Because of the presence of phenolic hydroxyl group, flavonoids can act as strong antioxidants by scavenging the reactive oxygen species (Cao *et al*., 2009). Condensed tannins are also known as proanthocyanidins, which are polymers formed by the condensation of flavans. Procyanidins are the most widely distributed condensed tannins in plants, which are derived from catechin or epicatechin and may contain gallic acid esters. It has been reported that the condensed tannins can act as primary antioxidants and free radical inhibitors by reacting with free radicals.

4. Conclusion

The present study revealed the presence of secondary metabolites in the coded drug 222 leaf ethanolic extract. TLC and HPTLC profiling of the extract were also carried out. The phytoconstituents such as total phenolics, total flavonoids and condensed tannin contents were estimated and the therapeutic effects of the extract were due to the presence of these compounds. Further detailed phytochemical research is warranted for the isolation and characterization of phytocompounds present in the extract.

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References

Cao G, Sofic E and Prior R L 2009. Antioxidant and pro-oxidant behaviour of flavonoids: Structure activity relationships. Free Radical Biol. Med. 22: 749-760.

Farnsworth N 2008. The Role of Ethnopharmacology in Drug Development. Bioactive Compounds from Plants. John Wiley & Sons. New Jersey, USA.

Harborne J B 1984. Phytochemical Methods. Chapman and Hall, London. 2: pp. 9-15.

Kale A, Gaikwad S, Mundhe K and Deshpande N 2010. Quantification of phenolics and flavonoids by spectrophotometer from *Juglaus regia*. International J. Pharma Bio Sci. 1 (3): 1-4.

Kunle O F, Egharevba H O and Ahmadu P O 2012. Standardisation of herbal medicines – A Review. Int. J. Biodiv. Conser. 4 (3): 101-112.

Makkar H P S and Becker K 1993. Vanillin-HCl method for condensed tannins: Effect of organic solvents used for extraction of tannins. J. Chem. Ecol. 19 (4): 613-621.

N M Krishnakumar, P G Latha, S Rajasekharan, S R Suja, Mathew Dan, B Sabulal and M Navas 2016 a. Preclinical studies of a medicinal plant (coded) based on Traditional Knowledge with special reference to Access and Benefit Sharing – Part 1. J. Trad. Folk Pract. 02, 03, 04 (1): 151-159.

N M Krishnakumar, P G Latha, S Rajasekharan, S R Suja, Mathew Dan, B Sabulal and M Navas 2016 b. Protective effect of coded plant (222) leaf extract against streptozotocin-induced diabetes in Wistar albino rats – Part II. J. Trad. Folk Pract. 04 (2): 37-47.

N M Krishnakumar, P G Latha, S Rajasekharan, S R Suja, Mathew Dan and M Navas 2017 a. Evaluation of the protective effect of coded plant leaf ethanolic extract

(222) against paracetamol-induced hepatotoxicity and oxidative stress in Wistar rats-Part III. J. Trad. Folk Pract. 05 (1): 8-15.

N M Krishnakumar, P G Latha, S Rajasekharan, S R Suja, Mathew Dan and M Navas 2017 b. Assessment of hepatoprotective activity of coded plant (222) leaf ethanolic extract against carbon tetrachloride-induced hepatotoxicity in Wistar rats-Part IV. J. Trad. Folk Pract. 05 (2): 111-120.

N M Krishnakumar, P G Latha, S Rajasekharan, S R Suja, Mathew Dan and M Navas 2018 a. Coded plant (222) leaf ethanolic extract ameliorates ethanol-induced liver damage and oxidative stress in Wistar albino rats-Part V. J. Trad. Folk Pract. 06 (1): 39-48.

N M Krishnakumar, P G Latha, S Rajasekharan, S R Suja, Mathew Dan and M Navas 2018 b. Hepatoprotective activity of coded plant (222) leaf extract in D-Galactosamine induced hepatotoxicity: Biochemical and histopathological analysis in experimental animals-Part VI. J. Trad. Folk Pract. 06 (2): 39-48.

Nagulendran K R, Velavan S, Mahesh R and Begum V H 2007. In vitro antioxidant activity of and total phenolic content of *Cyperus rotundus* rhizomes. E-Journal Chem. 4: 440-449.

Ofokansi K C, Esimone C O and Anele C K 2005. Evaluation of the in vitro combined anti-bacterial effects of the leaf extracts of *Bryophyllum pinnatum* (Fam.: Crassulaceae) and *Ocimum gratissum* (Fam.: Labiate). Plant Prod. Res. J. 9: 23-27.

Oliver S, Vittorio O, Cirillo G and Boyer C. 2016. Enhancing the therapeutic effects of polyphenols with macromolecules. Polymer Chem. 7: 1529-1544.

Raghava R D 1987. Statistical Techniques in Agricultural and Biological Research. Oxford & IBH Publishing Co., New Delhi.

Salah N, Miller N J, Pagange G, Tijburg L, Bolwell G P, Rice E and Evans C 1995. Polyphenolic flavonoids as scavenger of aqueous phase radicals as chain breaking antioxidant. Arch. Biochem. Bioph. 2: 339-346.

Singleton V L and Rossi J A 1965. Colourimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American J. Enol. Viticult. 16: 144–158.