RESEARCH ARTICLE

Standardization and Evaluation of Formulation Parameters of *Tinospora Cordifolia* Tablet

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ABSTRACT

Tinospora cordifolia a medicinal herb used in the Indian Ayurvedic system of medicine due to their health benefits.The aim of the present work is based on the standardization, Physiochemical & Phytochemical investigation including finger print analysis by HPTLC studies. The study also includes formulation and evaluation of solid oral dosage form (tablet) from plant extract. Several improved preformulation and formulation parameters had been studied for the standardization of solid oral dosage form of tablet in order to enhance its therapeutic efficacy. Besides this antioxidant activity of the prepared formulation was evaluated by DPPH free radical scavenging method and was found to be 59µg/ml followed by estimation of total flavonol and total phenolic content i.e 29.01mg/gm and 8.12 mg/gm of a sample respectively. Antibacterial activity of the prepared formulation was also evaluated by paper disc diffusion method. Quantification of tablet were done by HPLC method and total berberin content in 500mg of tablet was found to be 218ppm.

Keywords: *Tinospora cordifolia*, DPPH method, Paper disc diffusion method, formulation of the solid oral dosage form.

INTRODUCTION

Tinospora cordifolia also called Amrita, Giloy, Guduchi is widely used in Ayurvedic system of medicine "Rasayanas" to the immune system and the body resistance against infections [1]. It is a large, glabrous, deciduous climbing shrub belonging to family Menispermaceae is widely used in folk and Ayurvedic system of medicine it is referred as one of the most versatile rejuvenating herb. The species is widely distributed in India, Malaysia, Indonesia and Thailand. The Hindi name of the plant is Giloy, a Hindu mythological term that cites to heavenly elixir used by Celestial beings to stay off the aging and to stay young forever [2]. The stem of *T. cordifolia* is succulent with long filiform fleshy aerial roots from the branches. The bark is creamy white to grey, deeply left rosette like lenticels. The large numbers of compounds have been isolated from the aerial parts and roots of *T. cordifolia.* Flowers are yellow, growing in clusters from nodes. Fruits are drupes, turning red when ripe [3]. A variety of constituents have been isolated from different parts which includes berberin, tinosporaside, tinosporin, tinocordifolioside, cordifolioside A, cordifolioside B, isocolumbin, magnoflorine. It shows the presence of terpenoids, alkaloids, lignan, carbohydrates, bitters, steroids and glycosides. Different constituents like glycoside – giloin and a non-glucoside – gilenin and gilosterol have been found. The alkaloid tinosporin, tinosporic acid and tinosporol have been identified in the leaves. Tinosporidine and sitosterol isolated from stem, cordifol, heptacosanol and octacosonal from leaves a new furanoid diterpene – tinosporide isolated from stems [4]. One of the most important constituent present in stem of *T. cordifolia* is berberin, an isoqunoline alkaloid having molecular formula $C_{20}H_{18}N_{04}$ with molecular mass 336.36122 g/mol. It is yellow coloured alkaloid which shows strong yellow fluorescence under U.V light. It shows various pharmacological actions which enhances the therapeutic efficacy of this plant. Berberin a natural alkaloid recently used as an anticancer, antibacterial, antipyretic, anti-inflammatory, anti malarial, antidiabetic and in treatment of cardiovascular

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problems [5]. *T. cordifolia* a medicinal herb used in the Indian system of medicine due to their health benefits. In modern medicine it is used for the treatment of general weakness, fever, dyspepsia, dysentery, gonorrhoea, urinary diseases, viral hepatitis and anaemia more recently the immunomodulatory properties, antioxidant activity, antineoplastic activity, hypoglycemic activity, antipyretic activity, hepatoprotective activity, diuretic, anti-stress, antihyperglycemic, antidiabetic and anti tuberculotic activity were evaluated [6]. Hence regarding this solid oral dosage form of tablet is prepared with improved preformulation and formulation parameters which prove to be useful as an antioxidant as well as antibacterial activity.

MATERIALS AND METHODS

Plant material

Dried stems of *T. cordifolia* were collected in the month of January 2012 from Lahori gate. The plant stem was authenticated by Dr. H. B. Singh, Chief Scientist & Head Raw Materials Herbarium and Museum, NISCAIR, New Delhi vide Voucher Number NISCAIR/RHMD/Consult/-2011-12/1917/217 Dated January 06, 2012.

Instruments and Chemicals

Soxhlet apparatus, Round bottom flask, Heating mantle, Test tubes, China dish, Rota vapour, Silica crucible, Ashless filter paper (Whatman no.41), Petridish, Glass stoppered, Conical flask, Measuring cylinder, Muffle furnace (Thermotech), Hot air oven (NISCO), Grinding Mixer, Dessicator, Waterbath, Glass slides, Digital electronic weighing balance (Wenser, ISO 9001: 2000 certified), TLC chamber, HPTLC plates (Precoated aluminium sheet (10x10cm) with silica gel 60 F_{24} (Merk), Iodine chamber, Sprayer, HPTLC (CAMAG), Silica gel G for thin layer chromatography (Qualigens fine Chemicals), HCL (Merk), Phloroglucinol (Merk) and aniline (Qualigens fine chemicals), DPPH (Himedia Pvt Ltd), Ascorbic acid (Merk), Berberin (Sigma), FCR reagent (CDH Pvt Ltd), Ammonium acetate (Merk specialities Pvt Ltd), Sodium Hydrogen Orthophosphate (Fischer scientific India Pvt Ltd), Lactose (Merk specialities Pvt Ltd), Magnesium Stereate (CDH Labs).

Physiochemical studies

Physiochemical parameters like forgein matter, total ash, acid insoluble ash, water soluble ash, loss on drying, extractive values like water soluble and alcohol soluble extractive values were determined as per Indian Pharmacopoeia

Microscopic studies

Section cutting: Dried stem were taken and soaked in water for overnight. After that stem were cut into thin section and stained with Phloroglucinol & HCL**,** I_2 , KI and aniline blue on a clean glass slide and covered with a cover slip using glycerine and observerd under microscope.

Cork cells: Loosely arranged tangentially elongated which are broken at certain places due to the presence of lenticels. **Phelloderm** the inner to the cork is an inconspicuous layer which stains yellow on treatment with iodine and potassium iodide may be a lignified layer of phelloderm. **Cortex** next to phelloderm lies the cortex which consists of small compactly but irregularly arranged polygonal parenchymatous cells. These cortical parenchymatous cells contains abundant starch as they stains blue with Iodine and potassium iodide solution.

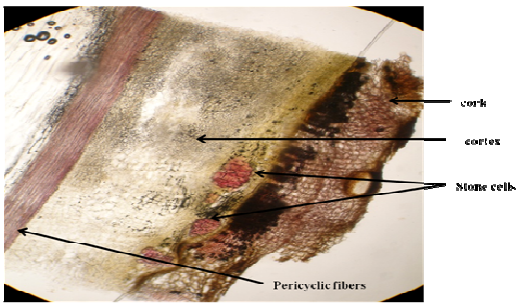

Fig 1: Transverse Section of *T.cordifolia*

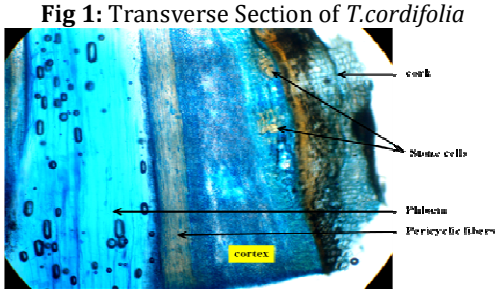

Fig 2: Transverse Section of *T.cordifolia* on Treatment with Phloroglucinol & HCL

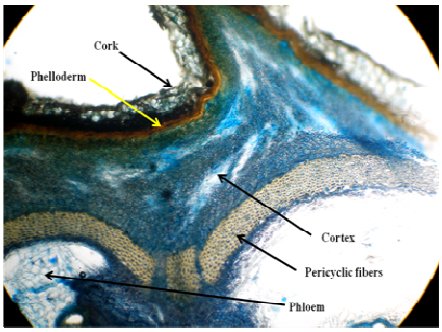

Fig 3: Transverse Section of *T.cordifolia* on Treatment with I_2 and KI

Xylem out of which phloem is present just below the pericyclic fibers and stained blue with differential stain I_2, KI and aniline blue. Vessels are cylindrical and have **Vascular tissue** consists of phloem and simple pits with annular thickenings. The strands of xylem are separated by 6-8 cell wide multiserate medullary rays. **Pith** consists of mainly thin walled cells containing starch granules as shown in figures given below.

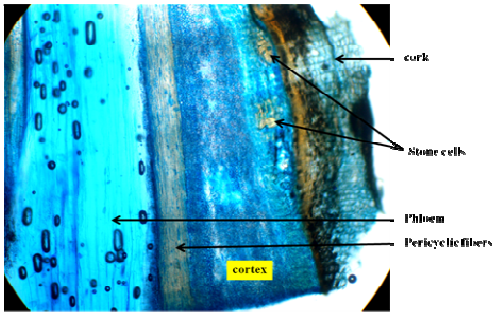

Fig 4: Transverse Section of *T.cordifolia* on Treatment with Aniline

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out on the methanolic extract of stem of *T.cordifolia* revealed the presence of a wide range of phytoconstituents including alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids, steroids and triterpenoids supporting the reason for its wide range of biological activities [7],[8].

Fingerprint analysis of methanolic extract of *T. cordifolia* stem by HPTLC

HPTLC fingerprint analysis was carried out on methanolic extract of *T. cordifolia* stem with solvent system i.e. Toluene: Ethyl acetate: Formic acid (5: 4: 1) using CAMAG HPTLC system consisting of linomat v spotting and scanner 3. The chromatogram obtained was studied under 254 nm, 366 nm [9].

Methodology:

100 mg of extract was weighed and dissolved in 5ml of methanol. The sample was subjected for sonication for 20 minutes and further be dissolved in 5ml. Samples are filtered and used for fingerprint analysis. Precoated aluminium sheet (10x10cm, Merck, Darmstadt, Germany) with silica gel 60 F254 of thickness 0.2 mm were used on sample which were applied in the form of band with the help of Linomat 3 applicator attached to HPTLC system which was programmed through winCATS, the software which were installed with the apparatus. 1ml of each of the sample was applied in the form of band of 3mm and chromatogram was developed in CAMAG twin through TLC chamber using solvent system chloroform: ethyl acetate: formic acid (4:5:2) by using anis- aldehyde sulfuric acid as a detecting agent. The developed chromatogram was then scanned using CAMAG TLC Scanner 3 at 254 nm and 366 nm using slit dimension 4x0.30m.

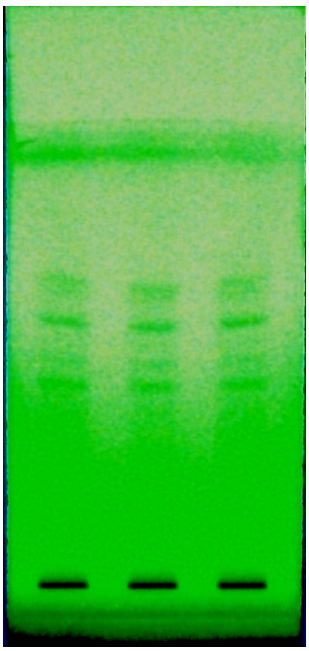

Fig 5: Image of Methanolic extract of *T. cordifolia* at 254nm

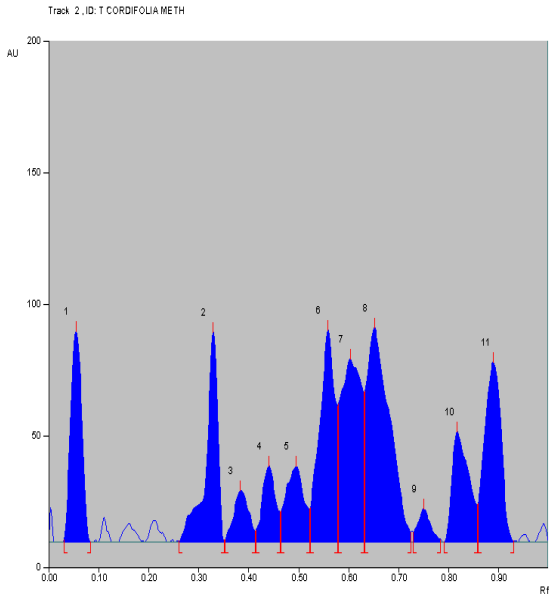

Fig 6: Finger printing analysis of *T. cordifolia* extract at 254 nm.

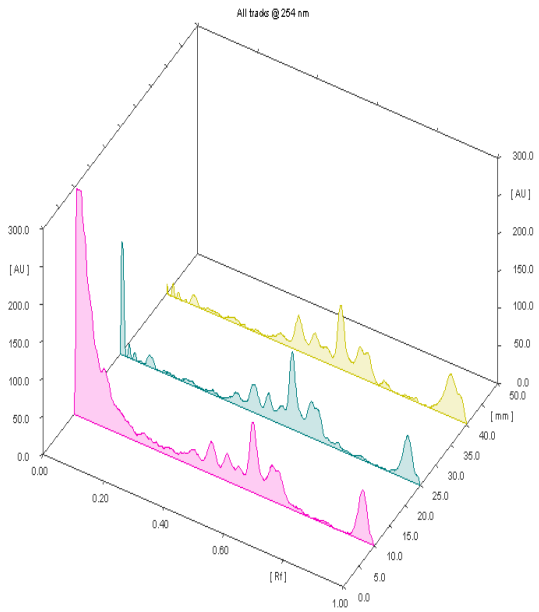

Fig 7: HPTLC Chromatographic profile (3D) of *T. cordifolia* extract at 254 nm

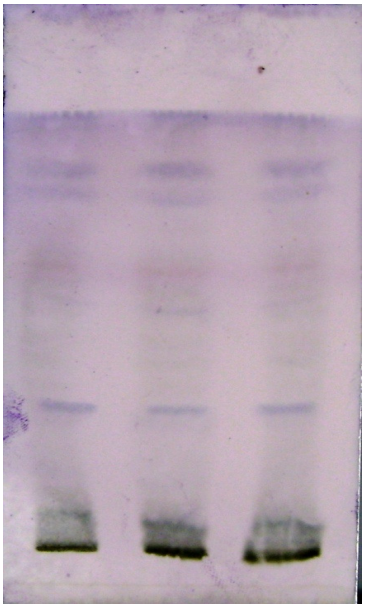

Fig 8: Image of *T. cordifolia* after spraying

Fig 9: Image of *T. cordifolia* at 366nm

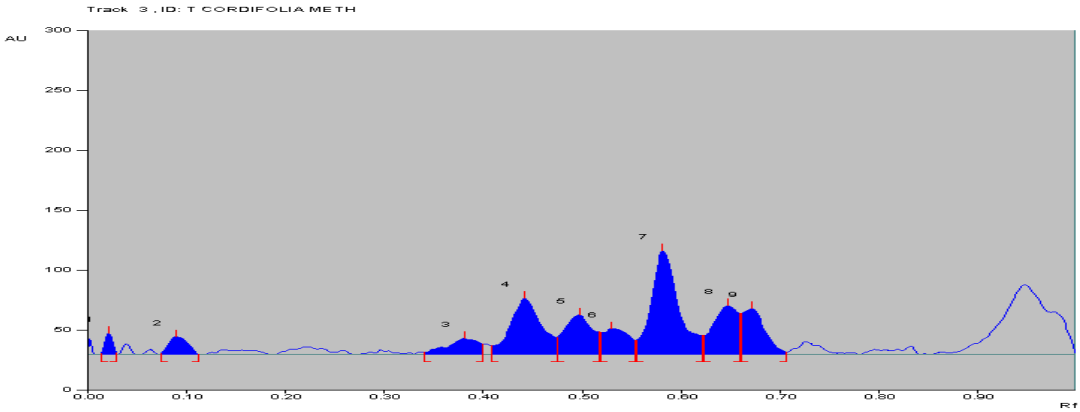

Fig 10: Fingerprinting analysis of *T. cordifolia* extract at 366 nm

Preparation of formulation

T. cordifolia tablets can be prepared by Wet granulation Method. Firstly the extract was allowed to pass through the sieve no. 20 along with lactose (90mg) and blend together in a mixing tray. After that wet mass was obtained by incorporation of starch solution (5%) to the dry mixture. The wet mass was made into dough and forced to pass through the sieve. The wet granules obtained were allowed to dry at the temperature of 45^0 C at the tray dryer for and 30 minutes. After that when granules are dried microcrystalline Na (60mg), cross caramalose sodium (0.9 mg), magnesium stereate (1%) and talc (1%) was added and they are passed through the sieve and milling was done. Hence the granules were ready for the compression to obtained tablets of about 500mg [10].

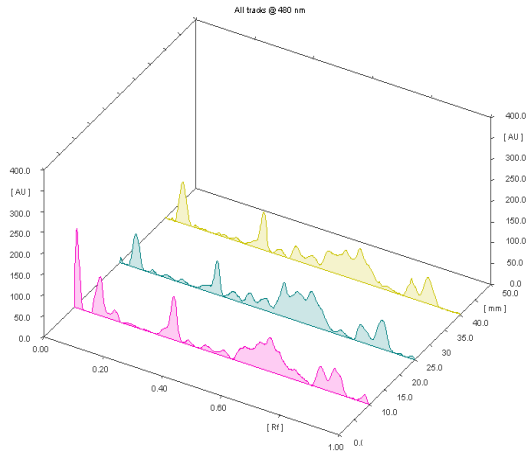

Fig 11: HPTLC Chromatographic profile 3(D) of *T. cordifolia* extract at 366nm

Preformulation and formulation studies

The granules were analysed for preformulation studies like bulk density, tapped density, [11],[12], Hausner's ratio, Carr's Index and angle of repose [13] Variuos formulation studies were studied like hardness and friability, disintegration time, dissolution rate of prepared formulation were performed. According to USFDA dissolution were carried out in 3 medium i.e 0.1 N HCl, 4.6 Acetate buffer and 6.8 phosphate buffer [14].

Quantification of prepared formulation by HPLC

Berberin content in prepared formulation were estimated by HPLC method. About 500 mg of the *T. cordifolia* tablet were weighed in triplicate and transferred it into volumetric flask and making it to the mark with methanol. 5ml of the solution were pipette out into clean 50 ml volumetric flasks and made to the mark with the mobile phase acetonitril: water (60:40), by using Column Reverse phase ODS, 250 × 4.6 mm, flow rate of about 0.5ml/min at the wave length of about 265 nm. A calibration curve of peak areas versus concentration of the standards was plotted. The berberin content in the oral dosage form was calculated using the regression equation of the best line of fit [15].

In vitro biological activity of prepared formulation

Antioxidant activity of *T. cordifolia* tablet by DPPH free radical scavenging assay. This assay is based on the reduction of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) which is a stable free radical with purple colour. Due to the presence of an odd electron it gives strong absorption maximum at 517 nm. Various dilutions of the extract and prepared formulation was prepared with methanol resulting in concentration in a range of 5µg/ml, 10 µg/ml, 20 µg/ml, 30µgm/ml, 40µ⍰g/ml, 50µg/ml, 100µg/ml, 150µg/ml. To each dilution was added 2 ml of the prepared DPPH solution in methanol. Control was prepared simultaneously consisting of 2ml methanol and 2ml of DPPH solution. The prepared dilutions were then left for colour development in the dark for 20 minutes. Finally the absorbance of control, Standard and Sample were measured at 517 nm against a methanol blank [16].

Total flavonol content

The total flavanol content in *T. cordifolia* tablet can be determined by Aluminium chloride colorimetric method . 10 mg of the sample was weighed and dissolved in 1 ml of 80% methanol using a vortex mixture (Touch Type) followed by adding to it 9 ml of methanol and finally mixing through sonicator to prepare a sample concentration of 1mg/ml. 0.5 ml of this solution was pipette out in a test tube to which was added 1.5 ml of 95% methanol, 0.1 ml of 10%

aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water .The final sample solution in the test tube was left to incubate for 30 minutes at room temperature. The sample was prepared in duplicate. Finally, the absorbance was measured at 415 nm against a reagent blank. The standard curve was obtained using rutin solution in the range of 10, 20, 40, 60, 80, 100µg/ml. The results will be expressed as mg rutin/g dry weight by comparison with rutin standard curve [17].

Total phenolic content

The total phenol content in *T. cordifolia* tablet was determined by using the Standard Folin-Ciocalteu method. 1.5ml of the sample was pipette out in a test tube to which 10.5 ml of distilled water was added followed by addition of 0.75ml of Folin-Ciocalteu reagent (FCR). This was left for incubation for 1-8 minutes at room temperature. Lastly to this 2.25ml of 20% Sodium carbonate solution was added and various dilutions were obtained in concentration 100µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500µg/ml, 600µg/ml, 800µg/ml,1000µ/ml and the dilutions were left to incubate for 2 hrs. Finally the absorbance was measured at 765 nm against a reagent blank. The Standard curve was obtained using gallic acid monohydrate. The total phenol content will be expressed as gallic acid equivalent to % w/w of sample [18].

Antibacterial activity

This method is based upon Paper disc diffusion method which involves diffusion of an antibiotic from a filter paper disc through the solidified culture media of a Petri dish used for study. Sample of *T. cordifolia* prepared formulation were prepared in different concentrations of about 5 µg/ml, 10 µg/ml, and 15 µg/ml with methanol. Paper disc impregnated with sample solutions as well as antibiotic solution (Ampicillin) were placed on to the surface of the solidified nutrient media of the plates with the help of a sterilised wire loop. After that the plates were incubated at 30^{0}C at BOD incubator. After 24 hours the diameter of zone of inhibition was measured for the evaluation of antimicrobial activity [19],[20].

RESULTS AND DISCUSSION

Physio-chemical Analysis

Physio-chemical parameters like total ash value, water soluble ash, aid insoluble ash, loss on drying and extractive value were shown in table 1.

Table 1: Physio chemical Parametres

S. No.	Parameters	% w/w values
1.	Forgein matter anaysis	0.12
2.	Ash values	
2.1	Total ash	6.12
2.2	Acid insoluble ash	1.08
2.3	Water soluble ash	1.22
3.	Loss on drying	4.1
4.	Extractive values	
4.1	Alcohol soluble extractive value	16.03
4.2	Water solule extractive value	20.7

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out on the methanolic extract of stem of *T.cordifolia* revealed the presence of a wide range of phytoconstituents phytoconstituents including alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids, steroids and triterpenoids as shown in table no. 2.

Table 2: Phytochemical screening

S. No.	Test name	Methanolic extract
1.	Alkaloids	+
2.	Carbohydrate	+
3.	Anthraquinone Glycosides	-
4.	Proteins And Amino acids	-
5.	Tannins And Phenolics	+
6.	Flvanoids	+
7.	Saponin	-
8.	Steroids	+

(+) Present; (-) Absent

Fingerprint Analysis of Methanolic Extract of *T. cordifolia* Stem at 254nm

HPTLC fingerprint analysis was carried out on methanolic extract of *T. cordifolia* stem with solvent system i.e. Toluene: ethyl acetate: formic acid (5: 4: 1)

using CAMAG HPTLC system. The R_f value of different compounds at 254 nm is shown in table no. 3.

Table 3: Finger print analysis of *T. cordifolia* at 254 nm.

S. No.	Solvent system	R_f values
1.	Toulene: Ethyl acetate: Formic acid (5:4:1)	0.38,0.44,0.50,0.53, 0.58,0.65,0.67 **(7 Spots)**

Fingerprint Analysis of Methanolic Extract of *T. cordifolia* Stem at 366nm as shown in table no. 4.

Table 4: Finger print analysis of *T. cordifolia* at 366 nm.

S. No.	Solvent system	R_f values
1.	Toulene: Ethyl acetate: Formic acid (5:1:15)	0.33, 0.38, 0.44, 0.49, 0.56, 0.60, 0.65, 0.75, 0.82, 0.86 **(10 Spots)**

Preformulation Studies for the Preparation of Tablet

Preformulation parameters like bulk density, tap density, Carr's index, Hausner's ratio and angle of repose were carried out. The granules showed excellent flow property as shown in table no. 5.

Table 5: Preformulation parameters

S. No.	Parameters	Result
1.	Bulk density	0.421 (gm/ml)
2.	Tapped density	0.512 (gm/ml)
3.	Hausner's ratio	1.21
4.	Carr's index	17.7
5.	Angle of repose	32.18 (θ)

Formulation Studies of Prepared Formulation

Formulation studies like hardness and friability, disintegration test and dissolution test were studied for the evaluation of tablet and shown in table no. 6

Table 6: Formulation parameters

S. No.	Parameters	Results
1.	Hardness	3.9 (kg/cm²)
2.	Friability	0.52 %
3.	Disintegration	13 minutes 20 Secs
4.	Dissolution	
a.	0.1 NHCl	76.54 %
b.	4.6 Phosphate buffer	73.1%
c.	6.8 Acetate buffer	75.8%

Quantification of 500mg of tablet by HPLC

A sharp peak of berberin was obtained by using mobile phase as acetonitrile: water (40:60). At a retention time of 5.6 at 265 nm. Berberin content in 500 mg of tablet was found to be 218 ppm as shown in table no. 7.

Table 7: Estimation of berberin content in 500 mg of prepared tablet

S. No.	Formulation	Berberin Content in 500mg of Tablet
1.	Tablet	218ppm

Antioxidant activity of prepared formulation

Antioxidant activity of prepared formulation was assessed by DPPH free radical scavenging assay. The formulation showed potent antioxidant activity with an inhibitory concentration (IC_{50}) of 59µg /ml . Ascorbic acid used as a standard showed an IC_{50} value of 6.12µg/ml as shown in table no.8

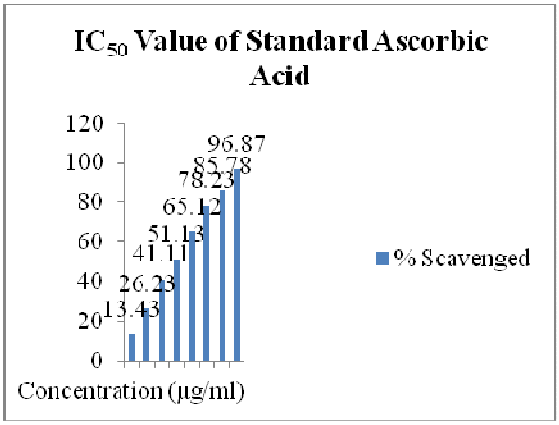

Fig 12: Standard curve of Ascorbic acid

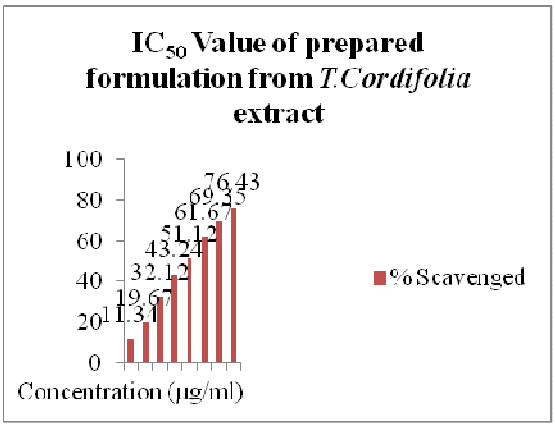

Fig 13: Free radical assay curve for tablet

Table 8: IC $_{50}$ value of prepared formulation

S. No.	Sample	IC_{50} value
1.	Std Ascorbic acid	6.12 µg/ml
2.	Tablet	59 µg/ml

Estimation of total flavonol content

The total flavonol content in prepared formulation was found to be 29.01 mg/gm as shown in table no.9. Rutin was used as a standard.

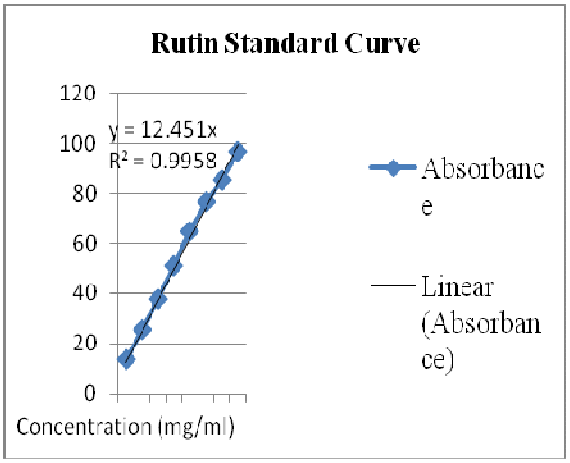

Fig 14: Standard curve for Rutin

Table 9: Result of Total Flavonol Content in *T. cordifolia* Tablet

S. No.	Formulation	Total Flavonol Content (mgRE/g of sample)
1.	Tablet	29.01

Estimation of total phenolic content

The total phenolic content in prepared formulation was found to be 8.12mg/gm as shown in table no. 10. Standard curve for Gallic acid was obtained.

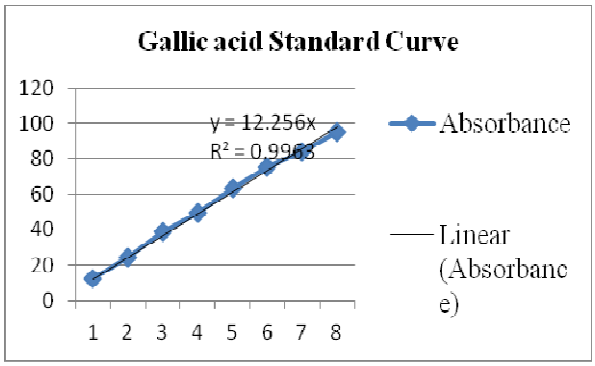

Fig 15. Standard curve for Gallic acid.

Table 10: Result of Total Phenol Content in *T. cordifolia* Tablet

S. No.	Formulation	Total Phenol Content (mgGAE/gof sample)
1.	Tablet	8.12

Antibacterial activity of Prepared formulation

The prepared formulation of *T. cordifolia* extract was screened for their antibacterial activity by paper disc diffusion method. Though the highest activity was observed against *E.coli* having Zone of Inhibition of 2.1 cm at the concentration of about 15µg/ml. Moreover, activity was quite reasonable and concentration-dependent in *B. subtilis* bacteria having Zone of Inhibition of diameter 1.2 cm at the concentration of about 15µg/ml . However, the standard drug ampicillin at the concentration of 5 µg/ml showed maximum activity against *B. subtilis* at the concentration of about 15 µg/ml having Zone of Inhibition of diameter of about 3.3cm as shown in table no.11.

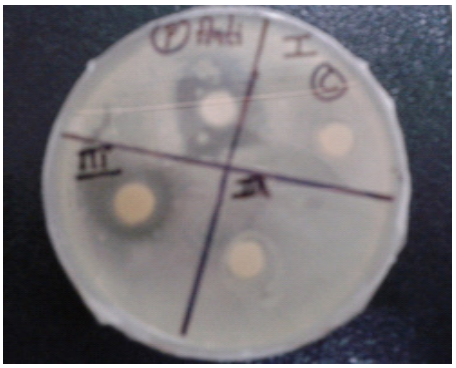

Fig 16: Zone of Inhibition of *E.coli*

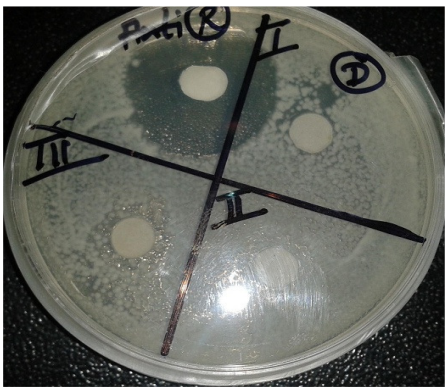

Fig 17: Zone of Inhibition of *B. subtilis*

Table 11: Results of Zone of Inhibition in *T. cordifolia* Tablet with Tetracycline by Paper disc diffusion method.

S. No	Formulation	Concentration(µg/ml)	*E.coli* (Zone of inhibition) (cm)	B.subtilis (Zone of Inhibition) (cm)
1.	Tablet	5 µg/ml	0	0
		10 µg/ml	0	0
		15 µg/ml	2.1	1.2
2.	Tetracycline	5µg/ml	3.2	3.3

CONCLUSION

The present study provides valuable information regarding the identification and authentication of the plant *T. cordifolia* along with the development of the solid oral dosage form with improved formulation parameters. Antioxidant rich plants serve as source of nutraceuticals that alleviate the oxidative stress and therefore prevent or reduce the onset of degenerative diseases. Therefore antioxidant activity of prepared formulation was evaluated by DPPH free radical scavenging assay. Antibacterial activity of the formulation was performed against *E.coli* and *B.subtilus* which clearly claimed its effects against several infections, inflammations and several other therapeutic benefits for human health. The present study justify the use of prepared formulation of *T. cordifolia* tablet in treatment of various infectious diseases and as a source of nutraceuticals in order to reduce oxidative stress with consequent health benefits. So further work could be done for the isolation and purification of important compounds from this plant which will allow the scientific community to utilise as an accessible alternative for the production of synthetic antibiotics. It shows many pharmacological activities as well. Hence this provides us a great scope of investigation regarding future prospects also.

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