Phytochemical Screening and Antioxidant Activity of *Curcuma longa*

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Abstract

Alzheimer is one of the most dangerous brain disorder the affect the elderly people. An underdiagnosed and undertreated illness is giving rise to a serious public health concern.The disease is due to the lack of cholinergic neurons in the brain, ultimately due to the deterioration of Acetylcholine (Ach) concentration. Acetylcholinesterase is an enzyme responsible for breakdown of Acetylcholine. Inhibition of AChE, is the most significant therapeutic target of AD therapy pathways. The present study revealed that the total weights of *Curcuma longa* (rhizome) used were 200 gm.In the pharmacognostic evaluation of *Curcuma longa* (rhizome) we determine total ash value , water soluble ash, acid insoluble ash , water extractive value, alcoholic extractive value , and loss on drying . Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by Reactive Oxygen Species. The determination of the total phenolic and flavonoids *Curcuma Longa*(rhizome) content of hydroalcoholic extracts were investigated. Recent investigations suggest that the plant origin antioxidants with free-radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis, and aging process. The antioxidant activity of plant extracts was determined by different *in vitro* methods such as the DPPH assay. In this investigation, the in-vitro antioxidant effect of *Curcuma Longa* were evaluated.

Keywords: *Curcuma Longa* , Anti- Alzheimer's Drugs, Herbal **,** *In-vitro activity*,Quantitative estimation, Anti-oxidant activity,Qualitative activity

1 Introduction

Alzheimer is one of the most dangerous brain disorder the affect the elderly people. An underdiagnosed and undertreated illness is giving rise to a serious public health concern..Over the past ten years, there has been a steadily increasing attempt to determine the cause of the condition and develop medication remedies[1]. Improved clinical diagnostic standards and better behavioural and cognitive disorders therapy are recent breakthroughs. Randomized, double-blind, placebo-controlled, parallel-group studies evaluating performance-based assessments of cognitive function, activities of daily living, and behaviour have been used to clinically evaluate symptomatic treatment that primarily focuses on cholinergic therapy. Patients with Alzheimer's

disease are advised to use cholinesterase inhibitors, such as donepezil, tacrine, rivastigmine, and galantamine, to address cognitive impairment[2].

The disease is due to the lack of cholinergic neurons in the brain, ultimately due to the deterioration of Acetylcholine (Ach) concentration. Acetylcholinesterase is an enzyme responsible for breakdown of Acetylcholine. Inhibition of AChE, is the most significant therapeutic target of AD therapy pathways. The disease is caused by a deficiency of cholinergic neurons in the brain, which in turn results in a decline in the concentration of acetylcholine (Ach). Acetylcholine is broken down by an enzyme called acetylcholinesterase. The most important therapeutic target in AD treatment routes is AChE inhibition.While irreversible AChE activities are linked to negative consequences, reversible, competitive, or incompetent AChE inhibitors are primarily used therapeutically. There is no indication that any drug can prevent or delay the onset of illness[3].

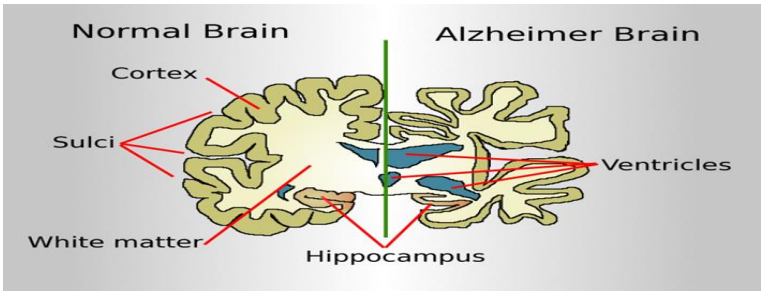


Figure 1: The regions, transmitters and circuit implicated in pathophysiology of Alzheimer[3].

1.1.Stages of Alzheimer's disease

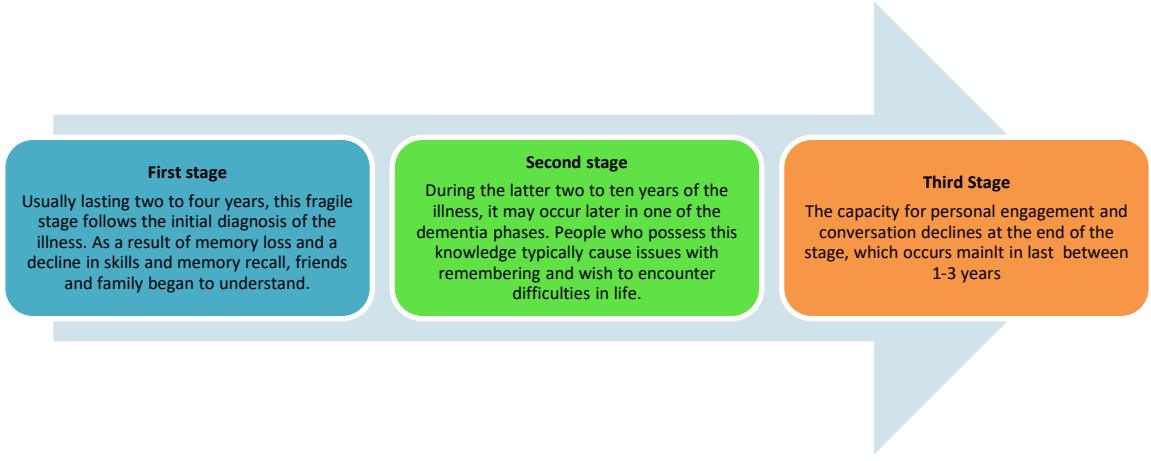


Figure 2 :Stages of Alzheimer's disease[4].

1.2 Etiology of Alzheimer's disease

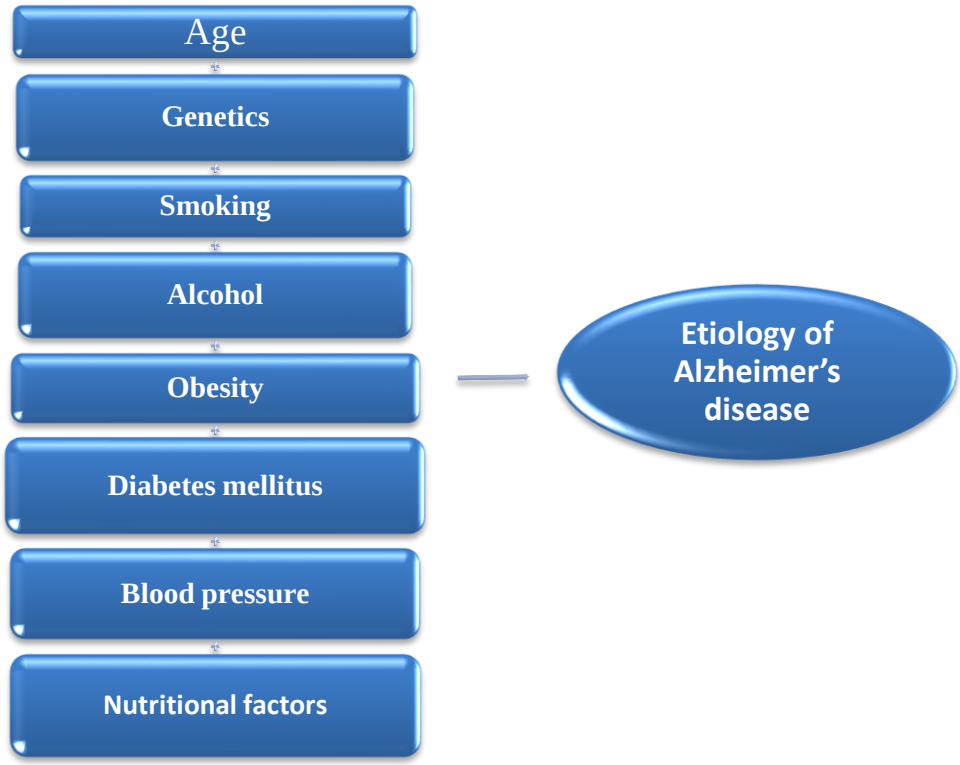


Figure 3: Etiology of Alzheimer's disease[5].

1.2 Anti- Alzheimer's Drugs

Table 1 : Classes of drug with its side effect[6].

Class of drugs	Examples of drugs	Side effects
Cholinesterase inhibitors	Tacrine, Donepezil, Rivastigmine	Nausea, Vomiting, diarrhea. Fatigue, muscle cramps and Hepatotoxicity
Cholinesterase inhibitors	Galantamine	Nausea, vomiting, Anorexia, Agitation and diarrhea
NMDA receptor antagonists	Memantine	Dizziness, headache, confusion, agitation and constipation.
Antioxidants	Garlic extract, Curcumin, Gink biloba, Vitamin E and Green tea	Hemorrhage, hematoma, headaches, GI distress and skin allergies [22]

1.3 Herbal plants used to treat Alzheimer

Table 2 : Herbal plants used to treat Alzheimer[7].

Name of Plant	Plant part used	Description
Camellia sinensis	Leaves	➢ Lowers Oxidative stress ➢ Improves memory
Curcuma longa	Roots or Rhizome	➢ Alpha secretase stimulatory effect.
Bugula neritina	Roots or rhizome	➢ Alpha secretase stimulatory effect by protein Kinase C
Physostigmavenenosum	leaves	➢ Beta secretase stimulatory effect.
Embeliaribes	Fruit	➢ Beta secretase stimulatory effect.
Rhodiola rosea	Resin	➢ cholinergic neurotransmission, improving coronary blood flow, decline in neuro-inflammation, apoptosis, and free radicals load
Taraxacum officinale	Whole Plant	➢ reduced LPS-mediated TNF-α, IL-1β generation, and activation of NF-kB

1.4 *Curcuma longa*:

Curcuma longa, commonly known as Turmeric, is a rhizomatous herbaceous perennial plant belonging to the Zingiberaceae family [(8)]. It originated in India and is widely cultivated in China, Sri Lanka, West and East Africa and other tropical countries. It is known as Jianghuang or Huangjiang in China. It is used in Chinese Traditional Medicine (TCM) for the treatment, prevention and management of various illnesses such as cancer, coughs, diabetes, Arthritis, diarrhoea, inflammation, psoriasis, hepatobiliary diseases, skin disorders, gastric ulcers and peptic ulcers [(9,10)]. It promotes blood circulation, removes stagnation, alleviates depression, and serves as a natural flavouring agent that strongly affects food's colour, taste and nature

2. Materials and Methods

2.1 Activity (*In-vitro* Anti-oxidant Activity)

2.1.1 DPPH Radical Scavenging Activity

2.1.1.1 Preparation of DPPH reagent

0.1mM solution of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared.

2.1.1.2 Preparation of Sample/Standard

Freshly 1 mg/ml methanol solution of extracts/standard was prepared. Different volume of extracts/standard (20 – 100µl) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly and absorbance was recorded at 517 nm after 30 minutes incubation in dark at room temperature.

2.1.1.3 Preparation of control

For control, 3 ml of 0.1mM DPPH solution was taken and incubated for 30 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm[11] **.**

Percentage antioxidant activity of sample/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

2.2 Pharmacognostical evaluation

2.2.1 Total ash value-

About 5 g of powdered parts were accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air dried powder**.**

$$\% \text{ Ash content} = \frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Weight of crucible + sample - Weight of crucible}} \times 100$$

2.2.2 Water soluble ash-

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water.The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted form the weight of total ash. The difference of weight was considered as water soluble ash. The percentage of water soluble ash was calculated with reference to air dried parts respectively[12].

$$\% \text{ Water soluble ash} = \frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Crude drug weight}} \times 100$$

2.2.3 Acid insoluble ash-

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug[12].

$$\% \text{ Acid insoluble ash} = \frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Crude drug weight}} \times 100$$

2.2.4 Alcoholic extractive value-

5g of powdered material was weighed into 250mL stopper conical flask containing 100mL of 90% ethanol and the stopper replaced. The flask and content was placed in a mechanical shaker for 6hrs and then allowed to stand for 18hrs. The mixture was filtered and 20mL of the filtrate was measured into an evaporating dish with a known weight, and evaporated to dryness. The

constant weight of the residue was gotten after drying in the oven at 1050 C for about 3 minutes[12]**.** The extractive value was calculated.

Alcohol soluble extractive value = Weight of reside /Weight of the drug ×100

2.2.5 Water extractive value-

The procedure was the same as above except that water used in place of 90% ethanol[12,13]**.**

Water soluble extractive value = Weight of residue /Weight of the drug × 100

2.2.6 Loss on drying

Place 2 to 6 g of the sample into a weighing bottle which has been accurately weighed, and weigh it accurately. Then, dry it at 105 o C for 5 - 6 hours and cool it in desiccators with silica gel. When the material is dried to a constant weight, the percent of loss on drying is determined[14]**.**

LOD % = Wt. of petridish + crude drug - After drying Wt. of petridish + sample × 100
Weight of crude drug

2.2.7 Soxhlet extraction:

Dried and powdered rhizome parts of aerial parts of *Curcuma longa were* placed in a thimble of Soxhlet apparatus separately. The extraction was carried out using a solvent system like 80 % hydroalcoholic at a temperature of 40-60ºC on the heating mantle for 8-10 hours. After the extraction process, the samples' extracts were filtered and concentrated to dry. Obtained extracts were evaporated using a rotary vacuum evaporator at 40°C. Extracts were collected in an airtight container[15]**.** The extraction yields of all extracts were calculated using the equation below:

Formula of Percentage yield = Actual yield X 100
Theoretical Yield

2.3 Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in Hydroalcoholic extracts of *Curcuma longa* using standard procedures[16]**.** The extracts were subjected to following tests:

2.3.1 Tests for carbohydrates:

2.3.1.1 Molisch test: To 1ml of extract, 2-3 drops of alcoholic α-naphthol solution was added. Conc. sulphuric acid was added along the side of the test tube. The appearance of purple ring at the junction of two liquids was observed, which confirms the presence of carbohydrates in the test samples.

2.3.1.2 Fehling's test: To 1 ml of extract, similar quantity of Fehling's solution A and B was added and heated on a water bath for few minutes. The development of brick red precipitate was observed.

2.3.1.3 Benedict's test: Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.

2.3.1.4 Barfoed's test: 1 ml of extract and Barfoed's reagent were mixed in a test tube and heated on water bath for 2 minutes. Red colour due to formation of cupric oxide indicates the presence of monosaccharide.

2.3.2 Test for alkaloids:

All the test extracts were first treated with dil. hydrochloric acid separately and filtered. The filtrate of all the test extracts was exposed to following tests:

2.3.2.1 Mayer's test: To 2-3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicates the presence of alkaloids.

2.3.2.2 Hager's test: To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow color precipitate indicates the presence of alkaloids.

2.3.2.3 Wagner's test: To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish-brown precipitate indicates the presence of alkaloids.

2.3.3 Test for flavonoids:

2.3.3.1 Lead acetate test: The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.

2.3.3.2 Alkaline reagent test: The extract was treated with few drops of sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicate presence of flavonoids.

2.3.4 Test for glycosides:

2.3.4.1 Borntrager's test: To 3 ml of extract, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates presence of anthraquinone glycosides.

2.3.4.2 Legal's test: 1 ml of extract was dissolved in pyridine. 1 ml of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red color indicates the presence of cardiac glycosides.

2.3.4.3 Keller-Killiani test: To 2 ml of extract, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of cardiac glycosides.

2.3.5 Test for protein and amino acids:

2.3.5.1 Biuret's test:T he extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of violet or pink colour indicates the presence of proteins.

2.3.5.2 Ninhydrin test: 3 ml of the extractwas heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino acids.

2.3.6 Test for saponins:

2.3.6.1 Froth test*:* 1 ml of extract was dissolved in 20 ml of distilled water and shacked for 15min in a graduated cylinder. Formation of persistent foam around 1cm layer was observed.

2.3.7 Test for triterpenoids and steroids:

2.3.7.1 Salkowski's test: The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layers turn red, sterol are present. Presence of golden yellow layer at bottom indicates the presence of triterpenes.

2.3.7.2 Libermann-Burchard's test: The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicate presence of steroids and formation of deep red color indicate presence of triterpenoids.

2.3.8 Test for tannin and phenolic compounds:

2.3.8.1 Ferric chloride test: Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

2.3.8.2 Lead acetate test: Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicates presence of phenolic compounds.

2.3.8.3 Gelatin Test: Into the distilled water some quantity of extract was dissolved. To this solution 2 ml of 1% gelatin solution containing 10% sodium chloride was added. Development of white precipitate depicts the presence of phenolic compounds.

2.4 Quantitative Phytochemical estimation-

2.4.1 Spectrophotometric Quantification of Total Phenolic Content: -

The total phenolic content of plant extract was determined using the Folin-Ciocalteu Assay. Hydro alcoholic extracts of *Curcuma longa* (0.2 mL from stock solution) was mixed separate in test tubes with 2.5 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 2 ml of a 7.5% Na_2CO_3 solution was added to the mixture and volume make up to 7 ml with deionized distilled water and mixed

thoroughly. The mixture was kept in the dark for 90 min at 25°C, after which the absorbance was read at 760 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (20 to 100µg/ml). The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample[17]**.**

2.4.2 Spectrophotometric Quantification of Total Flavonoid Content: -

The flavonoid content was determined using Aluminium chloride method [15]. In a 10 ml test tubes, 0.5 ml of Hydro alcoholic extracts *Curcuma longa* , 0.15 ml of $NaNO_2$ (5%) and 0.15 ml of $AlCl_3.6H_2O$ (10%) was mixed separate in test tubes. After 5 min, 2 ml of NaOH (4%) was added and volume up to 5ml with deionized distilled water. The solution was mixed well and the absorbance was measured against the reagent blank at 510 nm. The standard curve for total flavonoid was made using rutin standard solution (20 to 100µg/ml) under the same procedure as earlier described. The total flavonoid was expressed as milligrams of rutin equivalents per g of dried fraction[18]**.**

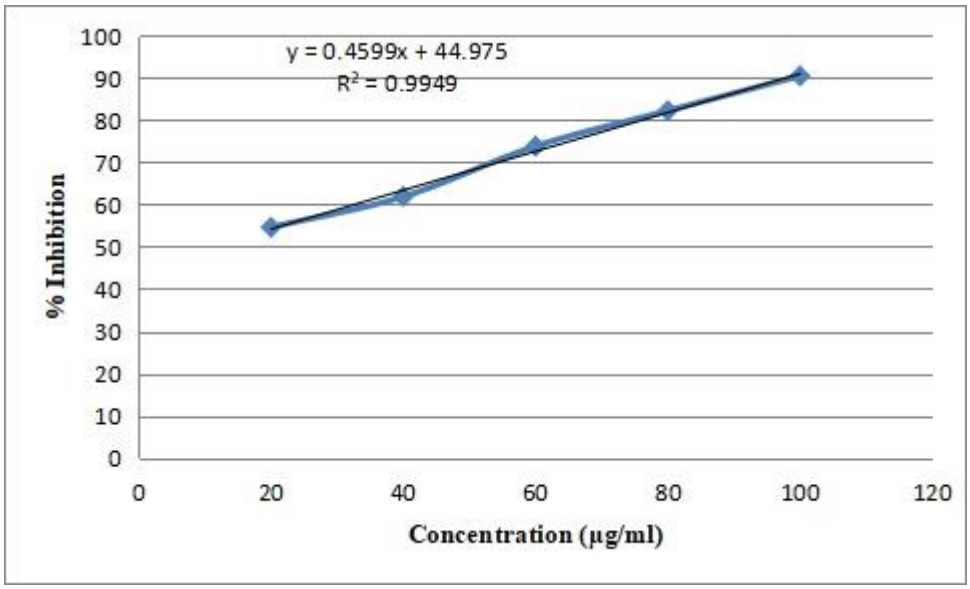
3. Results

3.1 Anti-oxidant activity

3.1.1 DPPH Assay

Table 3: DPPH radical scavenging activity of Ascorbic acid

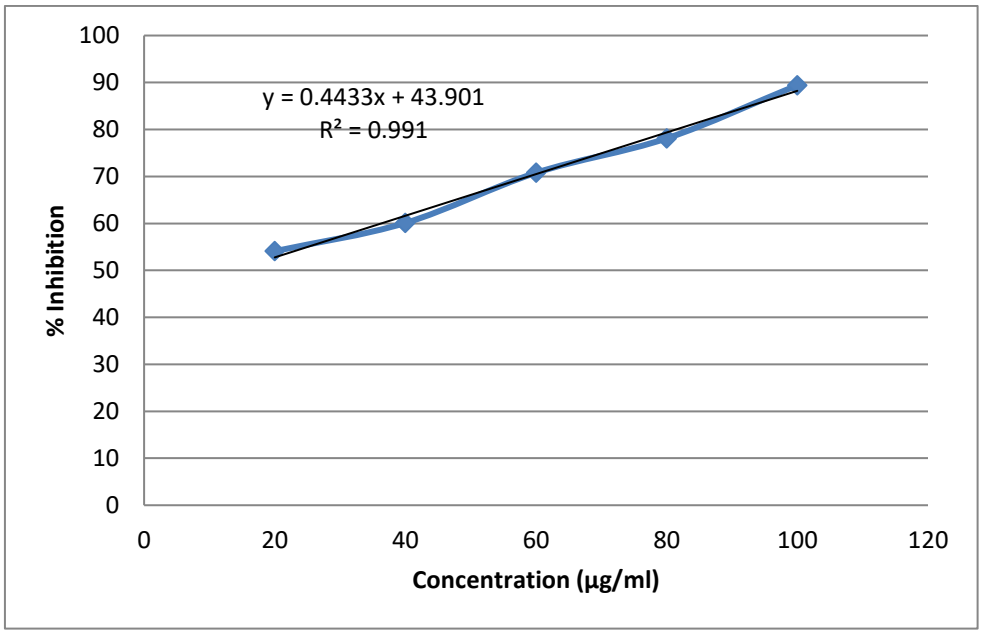
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.431	54.593
40	0.362	61.862
60	0.249	73.767
80	0.169	82.195
100	0.091	90.412
Control	0.9492	
IC50	**10.958**	



Graph 1 Graph represents the Percentage Inhibition *Vs* Concentration of Ascorbic acid

Table 4 : DPPH radical scavenging activity of Hydroalcoholic extract of ***Curcuma Longa***

Concentration (µg/ml)	**Absorbance**	**% Inhibition**
20	0.435	54.087
40	0.378	60.103
60	0.277	70.775
80	0.207	78.107
100	0.100	89.412
Control	0.9492	
IC50		13.769



Graph 1 Graph represents the Percentage Inhibition *Vs* Concentration of Hydroalcoholic extract of *Curcuma Longa*

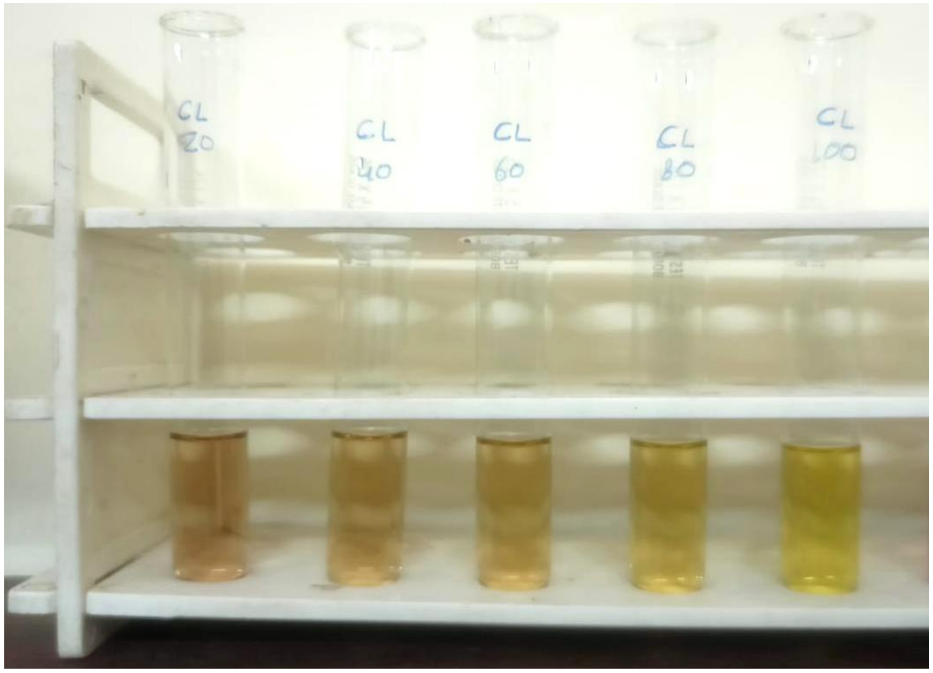


Figure 4: DPPH radical scavenging activity of Hydroalcoholic Extracts of *Curcuma Longa*

3.2 Pharmacognostical evaluation

Table 5 : Pharmacognostical evaluation of ***Curcuma Longa*** plant sample

Parameters	**Value in percentage (%)**
	Curcuma Longa
Total ash value	3.38
Water soluble ash	1.27
Acid insoluble ash	2.42
Water extractive value	0.29
Alcoholic extractive value	0.50
Loss on drying	0.20

3.3 Plant Collection

Table 6 : Plant collection of *Curcuma Longa*

Plant name	**Plant part used**	**Weight**
Curcuma Longa	Rhizome	200 gm

Figure 5 :Soxletation of *Curcuma Longa* with Hydro alcoholic solvent

Curcuma Longa (Hydro alcoholic)	 Figure 6

3.4 Percentage yield

Table 7: Percentage yield of *Artemisia vulgaris*

Plant name	**Solvent**	**Color of extract**	**Theoretical weight (gm)**	**Yield (gm)**	**% Yield**
Curcuma Longa	80% Hydro alcoholic	Dark Reddish to Brown	170	20.636	12.13

3.5 Qualitative Phytochemical Analysis of extracts of *Curcuma Longa*

Table 8: Qualitative Phytochemical Analysis of extracts of *Curcuma Longa*

S. No.	**Experiment**	**Result**
		Hydro alcoholic
Test for Carbohydrates		
1.	Molisch's Test	+
2.	Fehling's Test	+
3.	Benedict's Test	+
4.	Bareford's Test	+
Test for Alkaloids		
1.	Mayer's Test	+
2.	Hager's Test	+
3.	Wagner's Test	+
Test for Terpenoids		

1.	Salkowski Test	+
2.	Libermann-Burchard's Test	-
Test for Flavonoids		
1.	Lead Acetate Test	+
2.	Alkaline Reagent Test	+
Test for Tannins and Phenolic Compounds		
1.	$FeCl_3$ Test	+
2.	Lead Acetate Test	+
3.	Gelatine Test	+
Test for Saponins		
1.	Froth Test	+
Test for Protein and Amino acids		
1.	Ninhydrin Test	+
2.	Biuret's Test	+
Test for Glycosides		
1.	Legal's Test	+
2.	Keller Killani Test	-
3.	Borntrager's Test	+

Figure 7: Phytochemical study of Hydro alcoholic Extract of *Curcuma Longa*

3.6 Solubility determination of Hydro alcoholic extracts of ***Curcuma Longa*** -

Table 9: Solubility determination of Hydro alcoholic extracts of ***Curcuma Longa***

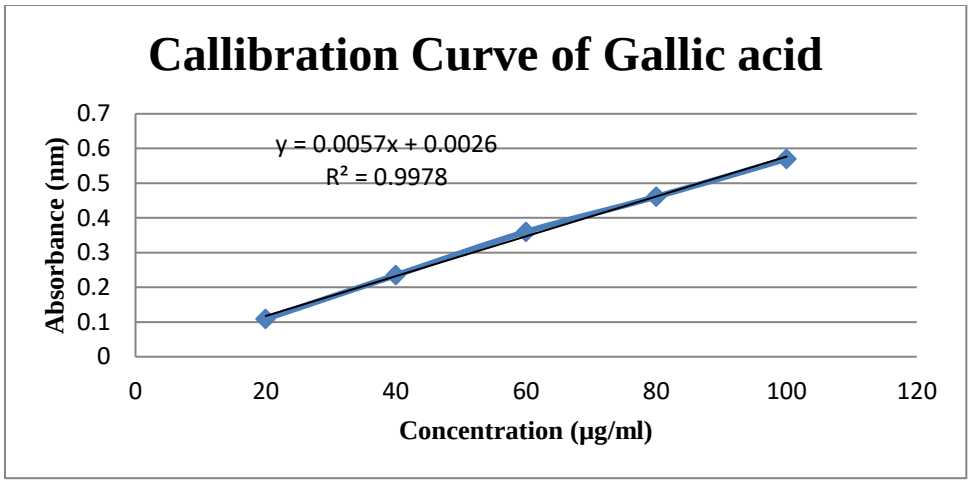
S. No.	Solvent	Result
1.	Water	Partially Soluble
2.	Ethanol	Soluble
3.	Ethyl Acetate	Sparingly Soluble
4.	DMSO	Soluble
5.	Petroleum Ether	Partially Soluble
6	Methanol	Soluble
7	Chloroform	Soluble
8	Acetone	Soluble

3.7 Quantitative Phytochemical analysis of extracts of *Curcuma Longa* -

3.7.1 Total Phenolic Content (TPC) Estimation

Table 10 Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	20	0.109
2.	40	0.235
3.	60	0.360
4.	80	0.461
5.	100	0.570



Graph 3 Graph represent standard curve of Gallic acid

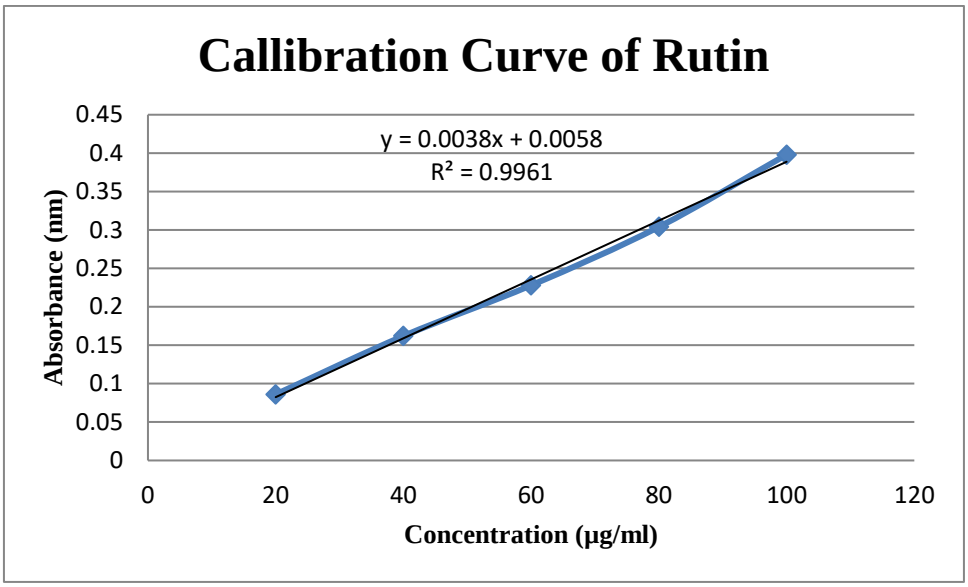
Table 11 Total Phenolic Content in *Curcuma Longa* extracts -

Total Phenolic content (mg/gm equivalent to Gallic acid)	
Extracts	***Curcuma Longa* (Hydro alcoholic)**
Absorbance Mean±SD	0.8806±0.004
TPC	175.72

3.7.2. Total Flavonoid Content (TFC) Estimation:

Table 12 Standard table for Rutin

S. No.	**Concentration (µg/ml)**	**Absorbance (nm)**
1.	20	0.086
2.	40	0.162
3.	60	0.228
4.	80	0.304
5.	100	0.398



Graph 4 Graph represent standard curve of Rutin

Table 1: Total Flavonoid Content in *Curcuma Longa* extracts -

Total Flavonoid content (mg/gm equivalent to Rutin)	
Extracts	*Curcuma Longa* **(Hydro alcoholic)**
Absorbance Mean±SD	2.1597±0.003
TPC	718.23

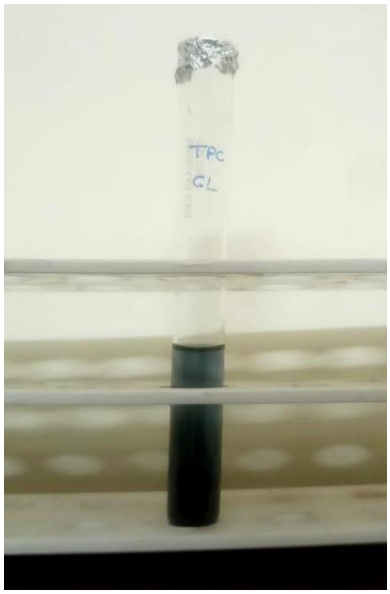


Figure 8: Quantitative Analysis (TPC) of Hydro alcoholic extracts of *Curcuma Longa*

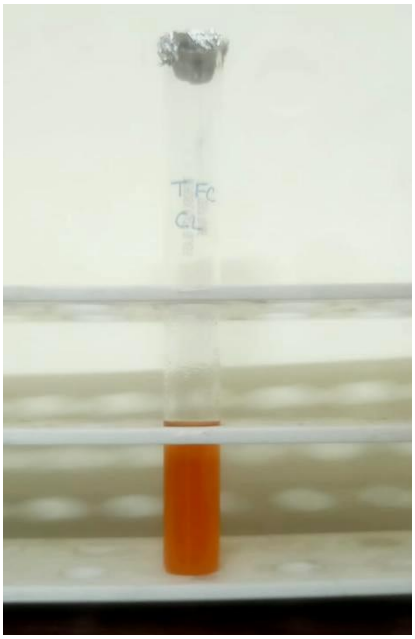


Figure 9 : Quantitative Analysis (TFC) of Hydro alcoholicextracts of *Curcuma Longa*

4 Summary and Conclusion

The present study revealed that the total weights of *Curcuma longa* plant (rhizome) used were 500 gm. After performing extraction of *Curcuma longa* plant (rhizome), the percentage yield of extracts in 80% hydroalcoholic solvent was found to be 12.13% (20.636 gm), respectively.

In the pharmacognostic evaluation of *Curcuma longa* plant (rhizome), total ash value (3.38), water soluble ash (1.27), acid insoluble ash (2.42), water extractive value (0.29), alcoholic extractive value (0.50), and loss on drying (0.20).

The hydroalcoholic extracts of *Curcuma longa* plant (rhizome) contain phytochemical constituents like carbohydrates, alkaloids, flavonoids, terpenoids, steroids, glycosides, proteins and amino acids, tannins, phenols, saponins, and compounds by phytochemical investigation with respect to chemical tests.The determination of the total phenolic content of hydroalcoholic extracts of *Curcuma longa* plant (rhizome) showed content values of 175.72, respectively. The total flavonoids content of the hydroalcoholic extracts of *Curcuma longa* plant (rhizome) showed content values of 718.23, respectively.

The antioxidant activity of plant extracts was determined by different *in vitro* methods such as the DPPH assay. In this investigation, the in-vitro antioxidant effect of *Curcuma longa* plant (rhizome) were evaluated. DPPH radical scavenging activity of Hydroalcoholic extracts of *Curcuma longa* plant exhibited percent inhibition 718.23 % and its IC_{50} value was found to be 13.769 μg/ml.

5 Reference

1. Schachter, Armand S., and Kenneth L. Davis. "Alzheimer's disease." *Dialogues in clinical neuroscience* 2.2 (2000): 91-100.
2. Förstl H, Besthorn C, Burns A, Geiger-Kabisch C, Levy R, Sattel A. Delusional misidentification in Alzheimer's disease: a summary of clinical and biological aspects. Psychopathology. 1994 Feb 10;27(3-5):194-9.
3. Scannevin RH. Therapeutic strategies for targeting neurodegenerative protein misfolding disorders. Current opinion in chemical biology. 2018 Jun 1;44:66-74.
4. Rowland D, Lyons B. Medicare, Medicaid, and the elderly poor. Health care financing review. 1996;18(2):61.

5. Grant WB, Campbell A, Itzhaki RF, Savory J. The significance of environmental factors in the etiology of Alzheimer's disease. Journal of Alzheimer's disease. 2002 Jan 1;4(3):179-89.
6. Guzior N, Wieckowska A, Panek D, Malawska B. Recent development of multifunctional agents as potential drug candidates for the treatment of Alzheimer's disease. Current medicinal chemistry. 2015 Jan 1;22(3):373-404.
7. Kim MiHye KM, Kim SungHoon KS, Yang WoongMo YW. Mechanisms of action of phytochemicals from medicinal herbs in the treatment of Alzheimer's disease.
8. Tung BT, Nham DT, Hai NT, Thu DK. Curcuma longa, the polyphenolic curcumin compound and pharmacological effects on liver. Dietary interventions in liver disease. 2019 Jan 1:125-34.
9. Ayman EL, Hassan SM, Mohamed AM, Mohammed HM. Tumeric or Curcuma longa Linn. InNonvitamin and Nonmineral Nutritional Supplements 2019 Jan 1 (pp. 447-453).
10. Kocaadam B, Şanlier N. Curcumin, an active component of turmeric (Curcuma longa), and its effects on health. Critical reviews in food science and nutrition. 2017 Sep 2;57(13):2889-95.
11. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. Journal of food science and technology. 2011 Aug;48:412-22.
12. Gami B, Parabia MH. Pharmacognostic evaluation of bark and seeds of Mimusops elengi L. Int J Pharm Pharm Sci. 2010;2(Suppl 4):110-3.
13. Egharevba HO, Kunle FO. Preliminary phytochemical and proximate analysis of the leaves of Piliostigma thonningii (Schumach.) Milne-Redhead. Ethnobotanical leaflets. 2010;2010(5):2.
14. Evans WC. Trease and Evans' pharmacognosy. Elsevier Health Sciences; 2009 May 27.
15. Alara OR, Abdurahman NH, Ukaegbu CI, Kabbashi NA. Extraction and characterization of bioactive compounds in Vernonia amygdalina leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques. Journal of Taibah University for Science. 2019 Dec 11;13(1):414-22.
16. Kokate CK. Practical Pharmacognosy. 3^< rd> ed. New Delhi. VPBN. 1991;3:107-11.
17. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC complementary and alternative medicine. 2012 Dec;12:1-2.
18. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis. 2002 Jul 1;10(3).