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RESEARCH ARTICLE OPEN ACCESS

Comparative pharmacognostic study and qualitative study of Curcuma and Amla

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ABSTRACT
In ethno medicinal practices, the traditional healers use the genus Curcuma for the treatment of various ailments Turmeric is a spice derived from the rhizomes of Curcuma longa, which is a member of the ginger family (Zingiberaceae). Rhizomes are horizontal underground stems that send out shoots as well as roots. Various parameters such as morphology, phytochemical profiles of the entire parts of the plant were studied and the salient diagnostic features are documented. Major chemical constituents, extractive values, physicochemical constants, and other features have also been recorded. Here, important chemicals like alkaloids, flavonoids, and amino acids were identified and isolated.
Amalaki or Indian goose berry is also known as King of all medicinal plants. It is most important drug in Indian traditional system, especially Ayurveda. It has occupied major place in Ayurvedic medicines. It is a small medium size tree. The leavesare feathery with small oblong pinnately arranged leaflets. The tree is characteristic greenish grey with smooth bark. Amalaki possesses the highest level of heat and storage stable vitamin C known to man. The study includes macroscopy, microscopy,preliminary phytochemicals screening.
Keywords: Curcuma longa, pharmacognostic standardization,Amalaki.

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I. PLANT PROFILE

1.1 *Curcuma longa L.* (Zingiberaceae)

Figure 1. Rhizomes of *C.longa*

Taxonomical Hierarchy:

Kingdom : Plantae
Division : Magnoliophyta
Order : Zingiberales
Family : Zingiberaceae
Genus : Curcuma
Species : *C. longa*

Part used: Rhizomes

Common Names

- Synonym : *Curcuma domestica* Valeton
- Sanskrit : Haridra
- Hindi : Haldi
- English : Turmeric
- Marathi : Halkund

Geographical Distribution:
The plant is native in China and East Indies and cultivated throughout India but predominantly in Madras, Bengal and Mumbai.

Botanical Description :
It is a tall perennial herb 2 to 3 ft high with short stem.

- **Rootstock:** large, ovoid, with sessile cylindrical tubers which is orange-colored inside.
- **Leaves:** very large, in tufts upto 1.2 m or more long, including the petiole which is oblong-lanceolate, tapering to the base.

- **Flowers:** autumnal spikes, 10-15 cm long; peduncle 15 cm or more, concealed by the sheathing petiole; flowering bracts are pale green in color; bracts of coma are tinged with pink.
- **Rhizomes:** Ovate, oblong, pyriform or cylindrical and short branched.Externally yellowish to yellow brown and internally yellow to yellow orange, waxy.

AyurvedicDescription :

Rasa	-	tikta, katu
Guna	-	lagu, rooksha
Veerya	-	ushna
Vipak	-	katu

Chemical Constituents:

The rhizome contains curcuminoids, volatile oil, sterols, sugars, starch and other polysaccharides. Curcuminoids are the principal coloring agent (6%) of which curcumin amounts 60%, with demethoxycurcumin, bis-demethoxycurcumin forming the rest. Volatile oil contains high amount of bisabolene derivatives along with borneol, camphene and α-phellandrene (Daniel, 2006). It also contains number of monoterpenes and sesqueterpenes mainly zingiberene, α and βtumerone, ar-tumerone. A Novel Sesquiterpenecurcumin L is isolated from *C. longa*.

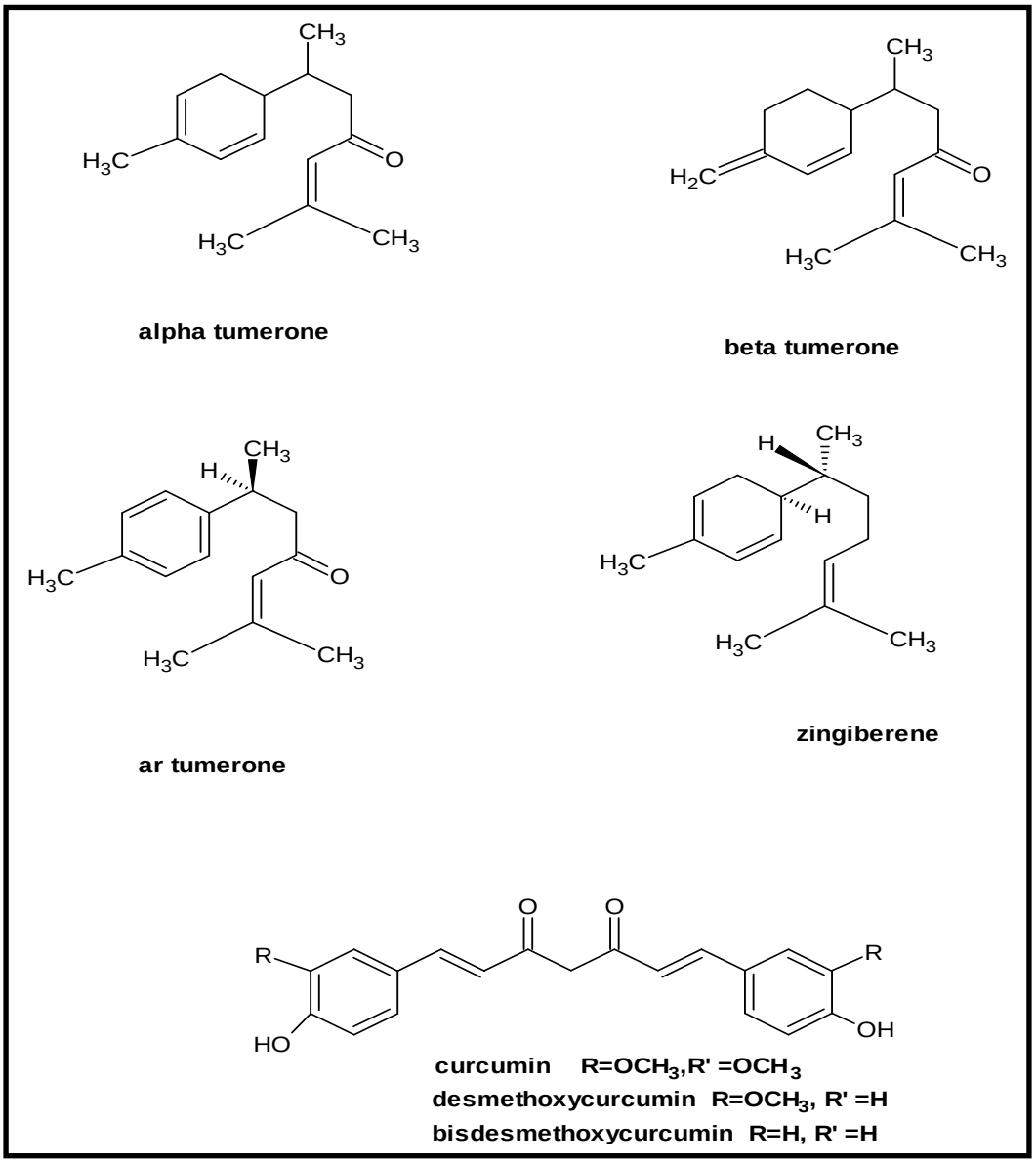


Figure 2. Chemical constituents from rhizomes of *C. longa*

Active Principle from Rhizomes of *C. longa*: Curcumin shows potent anti ulcer and antioxidant activity.

Uses:
Therhizomes arearomatic, carminative, jaundice, liver disorders and urinary diseases. It shows excellent anti-inflammatory, antiulcer, anticancer & antioxidant.

***1.2 P. emblica*(Phyllanthaceae)**

Figure 3. Fruits of *P. emblica*

Taxonomical Hierarchy :
Kingdom : Plantae
Division : Angiospermae
Class : Dicotyledonae
Order : Geraniales
Family : Phyllanthaceae
Genus : Phyllanthus
Species : *PhyllanthusemblicaL.*
Part used: Fruit
Common Names :

- Synonym : *Phyllanthus emblica L.*
- Sanskrit : Amalik
- Hindi : Amla
- English : Emblicmyrobalan
- Marathi : Awla

Geographical Distribution:
It is native to India, now growing wild as also cultivated. It is common in the mixed deciduous forests in India.
Botanical Description.
It is a deciduous small or middle sized tree with crooked trunk and spreading branches; bark is greenish grey, peeling off in conchoidal flakes; branchlets are glabrous or finely pubescent, 10-20 cm long.
Leaves: Subsessil, 10-13 by 2.5-3 mm long, light green in colour, narrowly linear, obtuse, imbricate when young, having appearance of pinnate leaves; stipules are ovate, finely acute.
Flowers: Greenish yellow in colour present in axillary fascicles on the leaf-bearing brachlets, with fimbriate bracts at the base.
Fruit: 1.3-1.6 cm diameter, fleshy, Surface is smooth, globose with 6 obscure vertical furrows, pale yellow in color, three 2-seeded crustaceous cocci. Seeds are 6, 3-gonous. Ripen from November to February, Nearly spherical or globular, wider than long and with a small and slight conic depression on both apexes. Fruit is 18-25 mm wide and 15-20 mm long, Mesocarp is yellow and endocarp is yellowish brown in ripened condition. In fresh fruit mesocarp is acidulous and in dried fruit it is acidulous astringent.
AyurvedicDescription :
Rasa - All rasas except lavan
Guna - lagu,rooksha
Veerya - sheeta
Vipak - madhura.

Chemical Constituents:
The fruit contain vitamin C (ascorbic acid, upto 2%), gallotannins (5%), carbohydrate (14%), phenolic acids, alkaloids, pectin and minerals. The phenolic acids include gallic, ellagic and phyllemblic acids and emblicol. The alkaloids present are phyllantidine and phyllantine, zeatin nucleotide and zeatinriboside.
The Benzenoid present are amlaic acid, corilagin3-6-di-O-galloyl-glucoseethyl gallate, 1,6-di-O-galloyl-β-D glucose1-di-O-galloyl-β-D glucose putranjivain A and digallic acid. The triterpenelupeol and Flavanoid kaempherol-3-O-β-D glucoside and quercetin-3-O-β-D glucoside are present.

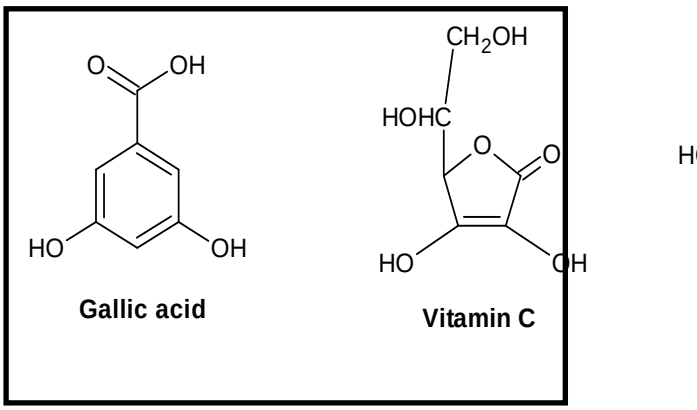


Figure 4. Chemical constituents from fruits of *P. emblica*

Active Principle from Fruits of *P. emblica*: Gallic acid, ascorbic acid shows potent anti-ulcer activity.

Uses:

The fruits are sour, astringent, bitter, acrid, sweet, cooling, ophthalmic, carminative, digestive, stomachic, laxative, alterant, aphrodisiac, diuretic, antipyretic and tonic. They are useful in vitiated conditions of tridosha, diabetes, cough, asthma, bronchitis, ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, skin diseases, leprosy, inflammations, anemia, jaundice, diarrhoea, dysentery, hemorrhage, cardiac disorders, intermittent fevers and greyness of hair.It exhibits antitumor, antiviral hypotensive, antibacterial &immunomodulatory properties.

II. MATERIALS AND METHODS

PLANT MATERIAL

Collection and procurement:

All parts of plant were collected from Ahmednagar district (M.S.). The parts of plant were dried under shade away from direct sunlight. The dried parts were cleaned and coarsely powdered in grinder and powder material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction.

Pharmacognostic studies:

- **Macroscopy:**

Organoleptic characters, extra feature and macroscopical details of all parts of plantwere carried out.

- **Evaluation of Physical Constants:**

1. Determination of foreign organic matter:

Five gm of air dried coarsely powdered drug was spread in a thin layer. The sample was inspected with the unaided eye or with the use of 6X lens. The foreign organic matter was separated manually as completely as possible. Sample was weighed and percentage of foreign organic matter was determined from the weight of the drug taken.

2. Determination of moisture content:

Accurately weighed glass stoppered shallow weighing bottle, and was dried. 2 gm of sample was transferred to the bottle and covered, the weight was taken and sample was distributed evenly and poured to a depth not exceeding 10 mm. Then loaded bottle was kept in oven and stopper was removed. The sample was dried to constant weight. After drying it was collected to room temperature in a desiccator. Weighed and calculated moisture content in terms of percent w/w.

3. Ash value:

Ash value is used to determine quality and purity of crude drug. Ash value contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium and calcium etc. sometimes inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects 'total ash value'. Such variables are then removed by treating with acid and then acid insoluble ash value is determined.

i. Determination of total ash:

Accurately weighed 2 gm of the air-dried crude drug was taken in a tarred silica dish and incinerated at a temperature not exceeding 450^0C until free from carbon, cooled in a desiccator and weight was taken.The process was repeated till constant weight was obtained. The percentage of ash was calculated with reference to air-dried drug.

ii. Water soluble ash:

The ash, obtained as per the method described above boiled for 5 minutes with 25 ml of water, filtered, and collected the insoluble matter in a Gooch crucible, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450^0C and weight was taken. The percentage of water-soluble ash was calculated with reference to air-dried drug.

iii. Acid insoluble ash:

The ash obtained as per method described above and boiled with 25 ml of 2M hydrochloric acid for 5 minutes, filtered, and collected the insoluble matter in a Gooch crucible or on an ash less filter paper, washed with hot water, ignited, and cooled in a desiccator and weighed. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

4. Extractive values:

Different extractive values like alcohol soluble extractive, water soluble extractive values were performed by standard method.

I. Determination of water-soluble extractive value:

Five gm of air dried coarsely powdered drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours and it was shaken frequently during first 6 hours and allowed to stand for 18 hours. Then it was filtered, 25 ml of the filtrate was evaporated in a flat shallow dish and dried at 105^0C and weighed. Percentage of water-soluble extractive value was calculated with reference to air-dried drugs.

ii. **Determination of alcohol-soluble extractive value:**

Five gm of air-dried coarsely powdered drug was macerated with 100 ml of ethanol of specified strength in a closed flask for 24 hours and it was shaken frequently during first 6 hours and allowed to stand for 18 hours. Then it was filtered, during filtration precaution was taken against loss of ethanol, 25 ml of the filtrate was evaporated in a flat shallow dish and dried at 105^0C and weighed.

Percentage of ethanol soluble extractive value was calculated with reference to air-dried drugs.

Phytochemical studies:

- **Extraction:**

Dried powdered rhizomes of *C. longa* and dried fruit powder of *P. emblica* were extracted by reflux distillation using 70% methanol. These extracts were vacuum dried to yield 13.39% w/w and 11.10% w/w of hydroalcoholic extracts, respectively.

- **Preliminary phytochemical tests:**

Preliminary phytochemical tests were performed as described by Khandelwal.

1) Test for steroids:

i) Liebermann-Burchard test

10 mg extract was dissolved in 1 ml of chloroform and 1 ml of acetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid from the side of the test tube. Formation of reddish violet color at the junction indicates the presence of steroids.

ii) Liebermann's test

To 2 ml of the extract a few ml of acetic anhydride was added and gentle heated. The content of the test tube were cooled and 2 ml of concentrated sulphuric acid was added from the side of the test tube. Development of blue color gave the evidence for presence of steroids.

iii) Salkowski test

One ml of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish brown color exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

2) Test for Glycosides:

i) Anthraquinone glycosides:

Borntrager's test

To 3 ml extract add dilute sulphuric acid, boil and filter. To the cold filtrate, add equal volume benzene or chloroform shake well. Separate organic solvent. Add ammonia, the ammonical layer turns pink or red indicates the presence of anthroquinone glycoside.

ii) Cardiac glycoside:

Keller-killani test

To 2 ml of extract, glacial acetic acid, one drop 5 % Ferric chloride and concentrated sulphuric acid was added. Presence of cardiac glycosides is indicated by formation of reddish brown color at junction of the two liquid layers and upper layer appeared bluish green.

3) Test for saponins:

Foam formation test

1 ml solution of the extract was diluted with 20 ml distilled water and shaken in a graduated cylinder for 15 minutes. The development of stable foam indicates the presence of saponins.

4) Test for flavonoids:

i) Shinoda test

To the extract 5 ml (95%) ethanol and few drops of conc. HCl and 0.5 g of magnesium turnings was added gives pink color indicates presence of flavonoids.

ii) Lead acetate test

Few drops of 10 percent lead acetate are added to the extract. Development of yellow colored precipitate confirms the presence of flavonoids.

5) Test for Alkaloids:

i) Dragendroff's test

0.1 ml dilute hydrochloric acid and 0.1 ml Dragendroff's reagent was added in 2 ml of extracts in test tube. Formation of orange brown precipitate indicates the presence of alkaloids.

ii) Mayer's test

2 ml of extract was taken in a test tube. 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff precipitate indicates the presence of alkaloids.

iii) Hager's test

2 ml of extract was allowed to react with 0.2 ml dilute hydrochloric acid and 0.1 ml of Hager's reagent. Formation of yellowish precipitate indicates the presence of alkaloids.

iv) Wagner's test

2 ml of extract was treated with 0.2 ml dilute hydrochloric acid and 0.1 ml of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

5) Test for Tannins and Phenolic compounds:

i) 5 % Ferric chloride test

5 ml of extract solution was allowed to react with 1 ml of 5 percent ferric chloride solution. Greenish black coloration indicates the presence of tannins.

ii) Potassium Dichromate test

2-3 ml of extract solution, mix with 2 ml of Potassium dichromate. The formation of red precipitate indicates presence of tannins.

iii) Bromine Water test

2 ml of extract solution mix with 2 ml of bromine water. Discoloration of bromine water indicates presence of tannins.

iv) Dilute Nitric acid test

2 ml of extract solution was allowed to react with few drops of dilute nitric acid solution. Formation of reddish to yellow color indicates the presence of tannins.

Thin Layer Chromatography

1. Detection of steroids:

Solvent system used

Toluene: Ethyl acetate (9:1)

Spray reagents

Anisaldehyde-Sulphuric acid reagent:

0.5 ml of anisaldehyde was mixed with 10 ml glacial acetic acid, followed by 85 ml of methanol and 5 ml of concentrated sulphuric acid, in that order. The developed TLC plate was sprayed with reagent, heated at 100^0C for 5-10 minutes. Color observed: blue, blue-violet or pink colored spots (Ayurvedic Pharmacopoeia of India, 2001; Stahl, 1969).

2. detection of Flavonoids:

Solvent system used

Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)

Spray reagents

Anisaldehyde-Sulphuric acid reagent:

Color observed: yellow green spots (WHO monographs 1999; AyurvedicPharmacopoiea of India, 2001; Stahl, 1969).

3. Detection of Saponins:

Solvent system used

Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)

Spray reagents

Anisaldehyde-Sulphuric acid reagent:Color observed: Orange-Red (Stahl et., 1969)

4. Detection of Tannins:

Solvent system used

Acetic acid: ethanol: Hexane: Formic acid (1:3:5:1)

Spray reagents

Ferric Chloride reagent: 5% $FeCl_3$ in 0.1N HCl. The developed TLC plate was sprayed with reagent. Color observed: bluish black spots **(**Wagner &Bladt, 1996).

5. Detection of Glycoside:

Solvent system used

Methanol: Water: Chloroform (35:10:65)

Spray reagents

Sodium nitroprusside reagent:

1.5 gm of Sodium nitroprusside is dissolved in 5 ml of 2N HCl, 95ml of methanol and 10 ml of 25% ammonium hydroxide solution are added and solution is filtered.

Color observed: Orange-red **(**Stahl et al., 1969).

6. Detection of Curcumin:

Solvent system used

Chloroform: Ethanol: GAA (94:5:1).

Color observed: Fluorescence visualization by uv.

7. Detection of Gallic acid:

Solvent system used

Chloroform: Ethyl acetate: Formic acid (5:4:1)

Spray reagents

Mixture of 1 volume of 1 in 100, 50% ethanol solution of ferric chloride and 1 volume of 1 in 100, 50% ethanol solution of potassium ferricyanide ,

Color observed: Dark-blue.

III. PHARMACOGNOSTIC STUDIES:

As per WHO guideline pharmacognostic study like macroscopy, physical parameters, and extractive values of all plant parts were studied.

Macroscopy

Morphological and Organoleptic properties of plant materials.

Table 1. Morphological and organoleptic characters of plant materials

Sr. No.	**Parameter**	**Part of Plant**	
		***C. Longa* (Rhizomes)**	***P. Emblica*(Fruits)**
1.	Color	Externally yellow & internally yellowish to orange	Greenish to yellow
2.	Odor	Aromatic	-
3.	Taste	Aromatic & bitter	Bitter
4.	Size	3 cm in diameter and 4 cm long	18-24mm wide at middle & 15-20mm
5.	Shape	ovate, oblong, cylindrical/pyriform and short branched	Spherical/globular, Slightly broader

Evaluation of physical constants

1. Foreign Organic Matter and Moisture Content

Table 2. FOM and moisture content of different parts of plant

Sr. No.	Parameter	Part of Plant	
		***C. Longa* (Rhizomes)**	***P. emblica*(Fruits)**
1.	Foreign organic matter	0.2% w/w	0.3% w/w
2.	Moisture content	7.5 % w/w	78 % w/w

2. Ash Value

Table 3. Ash values ofdifferent parts of plant

Sr. No.	Parameter	Part of Plant	
		***C. longa*(Rhizomes)**	***P. emblica*(Fruits)**
1.	Total ash	5.9 % w/w	6.6 % w/w
2.	Water- soluble ash	0.5 % w/w	4.9 % w/w
3.	Acid insoluble ash	0.5 % w/w	2.6 % w/w

3. Extractive Values:

Table 4. Extractive values of different parts of plant

Sr. No.	Parameter	Part of Plant	
		***C. longa*(Rhizomes)**	***P. emblica*(Fruits)**
1.	Alcohol soluble extractive value	15.8 % w/w	17.9 % w/w
2.	Water- soluble extractive value	16 % w/w	19.2% w/w

- **Phytochemical studies**

1. Preliminary Screening of Extracts

Table 5. Preliminary screening of extracts

Extracts	Color	Nature	Percentage Yield (% W/W)
*C. longa*hydroalcoholic extract	Yellowish brown	Sticky powder	16.42 %
*P. emblica*hydroalcoholic extract	Brown	Sticky powder	22.74%

2. Preliminary phytochemical tests

Table 6. Preliminary phytochemical tests ofdifferent parts of plant

Sr. No.	Chemical test	*C. longa*	*P. emblica*
1.	**Test for carbohydrate**		
	Molish's test	+	-
	Fehling test	-	-
	Benedicts test	+	+
	Barfoed's test	-	-
2.	**Test for Proteins**		
	Biuret Test	-	-
	Millions Test	+	+
3.	**Test for amino acids**		

	Ninhydrine test	-	-
4.	**Test for Steroids**		
	Salkowski test	+	+
	Libermann test	-	-
	LibermannBurchard reaction	+	-
5.	**Test for Glycosides**		
	Cardiac	-	-
	Anthraquinone	+	+
6.	**Test for Saponin**		
	Foam test	-	+
7.	**Test for Flavonoids**		
	Shinoda test	-	+
	Lead acetate test	+	+
8.	**Test for Alkaloids**		
	Dragondroff's test	+	-
	Mayer's test	+	+
	Hager's test	-	-
	Wagner's test	+	+
9.	**Test for Tannins and phenolic compounds**		
	5% Ferric chloride test	-	+
	Potassium dichromate test	-	+
	Bromine water test	+	+
	Dil. Nitric acid test	-	-
10.	**Test for Vitamin**		
	Vitamin C	-	+

+ indicates presence of constituents.
- indicates absence of constituents.

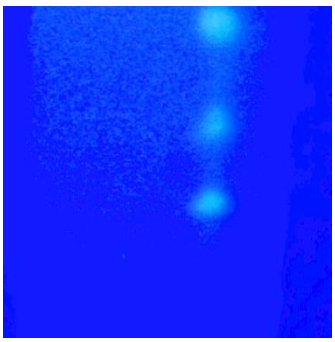
4. Thin Layer Chromatography

Thin layer chromatography technique carried out for separation, isolation, and identification of constituents present in the hydroalcoholic extracts.

1. **TLC For Curcumin**

Table 7. TLC of Curcumin

Sr. No.	Mobile Phase	Observation	Color of spot	Rf
1.	$CHCl_3$:Ethanol:GAA (94:5:1)	In uv chamber	Fluorescence	0.72



Curcumin

TLC for Gallic acid

Table 8. TLC for gallic acid

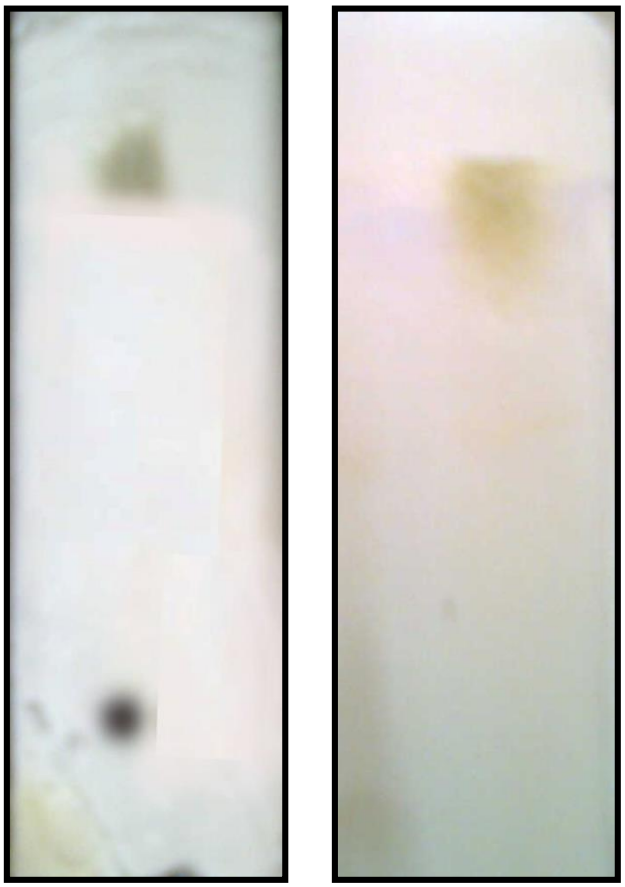
Mobile Phase	**Spraying reagent**	**Color of spot**	**Rf**
$CHCl_3$:Ethyl acetate:Formic acid (5:4:1)	$FeCl_3$ reagent	blue	0.51

Gallic acid

TLC for flavonoids and tannins:

Table 9. TLC for flavonoids and tannins:

Sr. No.	**Chemical constituents**	**Mobile phase**	**Spraying reagent**	**Color of spot**	**Rf**
1.	Flavonoid	N-butanol : acetic acid:H_2O(4:1:5)	Anisaldehyde – Sulfuric acid.	Yellowish green	0.81
2.	Tannin	Ethyl acetate: formic acid: acetic acid:H_2O (100:11:11:26)	5% $FeCl_3$ in 0.1N HCl	Black	0.80



Tannins **Flavonoids**

IV. SUMMARY AND CONCLUSION

From the obtained results, it can be concluded that:

- *C. longa* rhizome and *P. emblica*whole fruit extracts were used to prepare formulation because of their anti-ulcer and antioxidant activity.
- The total ash value was greater as compared to others. Water soluble ash was found higher than acid insoluble ash value.
- The results of extractive value showed significant results.
- Preliminary phytochemical tests of *C. longa* and *P. emblica* extracts was done. In this *C. longa* extract showed presence of flavonoids, steroids, glycosides, alkaloids, tannins, phenolic compounds while *P. emblica*extract showed presence of saponins, steroids, flavonoids, glycosides, alkaloids, tannins, and phenolic compounds.
- In TLC, extracts showed effective separation and presence of curcumin, flavonoid, tannin, and gallic acid.

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