ABSTRACT

Plants are the major source of drug in the modern as well as traditional system of medicine throughout the world. *Curcuma longa* belonging to the family Zingiberaceae, is a medicinally important perennial herb distributed throughout the tropical and sub-tropical regions of the world from sea level to 1200 m. Therapeutically, *C. longa* has been used for curing various diseases such as asthma, anemia, chronic bronchitis, fever, dysentery, cough, diabetes, diarrhea, eye disease, hepatitis, hysteria, indigestion, itching, leprosy, liver disorder, menstrual disorder, peptic ulcer, small pox, chicken pox, tonsillitis, urinary infection etc. The objective of the study was phytochemical screening of *C. longa* for highlighting the traditional uses and pharmacological properties of its rhizomes. The rhizomes were collected from Pokhara Metropolitan -3, Nadipur, Nepal in November 2019. The collected rhizomes were washed thoroughly, cut into pieces, shaded dried completely, grinded into fine powder, extracted by Soxhlet extractor, evaporated the extract to get dark orange residue. Phytochemical screening was conducted by using standard methods. Phytochemical analysis of methanolic and ethanolic rhizome extract of *C. longa* showed that it contained alkaloids, steroids, tannins, flavonoids, carbohydrates, cardiac, glycosides, phytosterol, anthocyanin, emodins, diterpenes, leuco anthocyanin, anthroquinone, chalcones, phenols, coumarin and phlobatannin in methanolic extract but not in ethanolic. Previous pharmacological studies on *C. longa* showed that it possessed antiviral, antifungal, anticancer, antidepressant, antimalarial, antimicrobial, antioxidiant, antiplatelet, antibacterial, antivenum, antimitagenic and anticarcenogenic activities. Further phytochemical analysis using solvents of different polarity is necessary to identify much more phytochemical constituents for highlighting pharmacological and traditional medicinal properties.

Key words: Curcuma, medicinal chemistry, phytochemistry, traditional medicine

INTRODUCTION

Medicinal plants contain compounds or synthesize metabolites that can be used for producing useful drugs (WHO, 2002). The world health organization estimated that 80% of people worldwide rely on herbal medicines partially for their primary health care (IUCN, 2011). Plants are the major source of drug in the modern as well as traditional system of medicine throughout the world. Over 60% of all pharmaceuticals are plant based (Jain* et al.*, 2007). *Curcuma longa* commonly known as turmeric (Haledo in Nepali and Haldi in Hindi) belongs to the family Zingiberaceae. It is extensively cultivated for its rhizomes. It is a perennial herb distributed throughout tropical and sub-tropical regions of the world including India, Pakistan, Bangladesh, Sri Lanka and Nepal. Nepal is rich in culture and biodiversity, therefore, different traditional systems of medicine have been in
practice. Turmeric has been used in Nepal and India as a drug in Ayurvedic and Unani system of medicine from the time immemorial to treat wide range of ailments. *Curcuma longa* is cultivated from sea level up to 1200 m at temperature 20-30°C with good rain fall, warm and humid climate. Turmeric cultivated in the hill is reported to be of a better quality than that raised in the plans. The light green leaves of the plant are large (up to 1.2 m long and 8-12 cm wide) oblong, narrowed to the base (Hooker, 1990). The individual flowers are yellowish white or yellow in colour. The fruit is globular capsule (Thomas, 2000), but it is rare (Anonymous, 2004). The rhizomes are fleshy, branched with bright orange to yellow (Ross, 2001). The primary rhizomes are ovate or pear-shaped are known as ‘bulb’ or ‘round’ turmeric while secondary rhizomes are more cylindrical which are known as ‘fingers’ and contain more yellow colouring matter than the bulb variety (Evan, 2002). *Curcuma longa* has an aromatic odour and a warm somewhat bitter taste. It is commonly used as a preservative, food additive, and food colouring agent. Large quantities of turmeric are used in the preparation of curries and sauces. It forms an integral component of diet (Ahmad et al., 2010). In addition to its use as spices and pigments, turmeric has been used in Nepal and other Asian countries for medical purposes for centuries. Traditionally, *C. longa* has been used as aromatic stimulant, tonic, blood purifier, antiparasitic, in sprains, wounds, injuries, chest and abdominal distension, mucous discharge, conjunctivitis etc (IUCN, 2000). Its rhizome is traditionally, used as blood purifiers, tonic to brain and heart, to treat leucoderma, piles, bronchitis, asthma, tumours, tuberculosis, glands on the neck, enlargement of spleen, to check leucorrhal and gonorrhoea discharge It is used as preservative, colouring matter and has wide range of medicinal and pharmacological applications (Chanda et al., 2019). It is used as colouring matter in pharmacy, confectionery, food industry, for dying wool, silk, cotton and in combination with other natural dyes to get different shades. Turmeric is used for the formation of certain cosmetic soaps particularly effective in skin problems and to remove unwanted hairs (Anonymous, 2004). Therapeutically, it has been used for curing in various diseases such as asthma, anemia, chronic bronchitis, chronic dysentery, chronic fever, conjunctivitis, cough, diabetes, diarrhea, dislocation of joint, eye disease, gonorrhea, hepatitis, hysteria, indigestion, itching, leech bite, leprosy, liver disorder, loss of appetite, menstrual disorder, parasitic disease, peptic ulcer, small pox, chicken pox, skin disease, tonsillitis, toothache, urinary infection, urine pain, vaginal discharge, weakness of eye sight etc. (Ahmad et al., 2010). In Ayurveda, it is recommended in elevated condition of *Kapha* and *Pitta*. Turmeric is an important component in religious ceremonies and offerings (Dastur, 1970). Turmeric contained an essential oil, an alkaloid, starch grains, yellow matter curcumin and other curcuminoids, turmeric oil, turmerol, coporic acid as free acid, veleric acid as a combined acid (Jain et al., 2007). Several studies showed that turmeric inhibits the growth of several different types of cancer cells. Curcumin can bind with heavy metals such as cadmium and lead there by reducing the toxicity of these heavy metals.

The phytochemical analysis of the methanolic extract of *C. longa* showed the presence of tannins, alkaloids, saponins, flavonoids, terpenoids, cardiac glycosides, steroids, phytoesterol and phenol (Sawant and Godghate, 2013). The photochemical constituents present in ethanol extract of rhizomes of *C. longa* are alkaloids, saponins, tannins, anthocyanin, emodins, flavonoids, diterpenes, phlobatannin,
leucoanthocyanin, anthroquinone, chalcones, cardiac glycosides and carbohydrate. The phytochemical constituents present in the *C. longa* have been shown to possess anti-oxidant (Anto *et al.*, 1994), anti-allergic activity, antimicrobial activity (Negi *et al.*, 1999), antibacterial activity (Singh *et al.*, 2002), antiulcer activity, antivenom activity, antifertility and antispermatic activity (Bhagat *et al.*, 2001), antidepressant activity (Xu *et al.*, 2002), antiinfective (Lee *et al.*, 2007), antiemetic activity, anti-inflammatory, anticancer, anticarcinogenic, antimutagenic, antifungal, antiviral properties (Chattopadhyay, *et al.*, 2004). Yellow colouring matter curcumins and other curucinoids (diarylheptanoids) and essential oils are the major bioactive ingredients (Sabale *et al.*, 2013). Arutselvi *et al.*, (2012) has reported antimicrobial activity from leaves and rhizomes of *C. longa*.

Nowadays, the interest in using natural sources or medicinal plants is increasing worldwide due to their safety, efficiency, cultural acceptability and lesser side effect as compared to synthetic drugs. Recent trends in *C. longa* Linn has been studied by Jain *et al.* (2007) to review on a description of its various pharmacological actions studied earlier and in the recent times. The objective of the research was phytochemical screening of *Curcuma longa* for highlighting its traditional uses and pharmacological properties. Phytochemical constituents of *Curcuma longa* available in Pokhara Metropolitan City-3, Nadipur, Nepal were phytochemically analysed in chemistry research laboratory of Babasab Bhimrao Ambedkar Bihar University, Muzaffarpur, India.

**MATERIALS AND METHODS**

**Plant material and extract preparation**

Fresh plants of *C. longa* were collected with leaf and rhizomes from Pokhara metropolitan city-3, Nadipur, Kaski, Nepal in November 2018. The plant and rhizomes were authenticated by experts. Extraction and phytochemical screening was carried out in Research Laboratory of Department of Chemistry, Babasab Bhimrao Ambedkar Bihar University, Muzaffarpur, India. The rhizomes were washed thoroughly, cut into small pieces, shaded dried completely for a week at room temperature and grounded well into fine powder. The powder was stored in a clean closed air tight container with proper labelling until further use. 40 g of dried powder of turmeric were placed in the thimble of Soxhlet apparatus. 150 ml of a solvent (methanol or ethanol) was used at a time. The extraction was continued till clear solvent was seen in the thimble. The extract was separated and dried in a digital water bath till a dark orange residue was obtained. The rhizome powder of turmeric was extracted by using a specific solvent separately in an operation. The extracts were kept in a refrigerator at 4°C for further use.

**Phytochemical Analysis**

The test sample extract was subjected to phytochemical analysis in order to find out the presence or absence of phytochemical constituents. The phytochemical tests were employed for alkaloids, steroids, tannins, saponins, flavonoids, phenols, carbohydrates, cardiac glycosides, phytosterol, anthrocyanin, coumarin, emodins, deterpenes, phlobatannin, locoanthocyanin, anthroquinone, chalcones by adopting standard procedures.

1. **Detection of alkaloids**

**Wagner’s test:** 20 mg of turmeric extract was dissolved in 2ml of specific solvent with few drops of 1% HCl and heated and then cooled. On adding Mayer's reagent (Potassium mercuric iodide) dropwise, the yellow or brown reddish precipitate was indicated that the presence of alkaloids (Ogunlowo *et al.*, 2013).
2. Detection of saponins

Foam test: 40 mg of turmeric extract was mixed with 20 ml of distilled water and shaken vigorously for few minutes. Formation of thick foam layer was indicated that the presence of saponins.

3. Detection of steroids

Liebermann-Burchard test: 20 mg of extract was dissolved in 5 ml of solvent (chloroform) containing equal volume of acetic anhydride and few drops of concentrated H$_2$SO$_4$ was added along the side of test tube. The development of dark green coloration indicated the presence of steroids.

4. Detection of terpenoids

Liebermann-Burchard test: 20 mg of extract was dissolved in 5 ml of organic solvent (ethanol). To it 5 ml of acetic anhydride was added followed by addition of 1-2 drops of conc. H$_2$SO$_4$, change of colour from pink to violet/red-violet indicated that presence of terpenoids (Ogunlowo et al., 2013).

5. Detection of tannins

FeCl$_3$ Test: 20 mg of extract in 5 ml of solvent (distilled water) was treated with few drops of 1% FeCl$_3$ solution. Formation of green or blue green colour indicated the presence of tannins.

6. Detection of phenol

Ferric chloride test: 20 mg of extract in 5 ml of distilled water was treated with few drops of alcoholic ferric chloride solution and observed for the formation of bluish-black colour (Sawant and Godhate, 2013). This test was similar with the test of tannin.

7. Detection of flavonoids

Alkaline reagent test: Extract was treated with 10% NaOH solution, formation of intense yellow colour which changes to colourless on adding dilute HCl was indicated the presence of flavonoids.

Lead acetate test: On adding few drops of lead acetate on few mg of extract, yellow colour precipitate indicated the presence of flavonoids (Evan, 2002).

Zn test: Formation of red colour on treating 2 ml extract with Mg or Zn dust and concentrated HCl indicated the presence of flavonoids (Shrestha et al. 2016).

8. Detection of protein

Biuret’s test: A mixture of 2 ml of Biuret reagent and 2 ml of extract was shaken well and warmed in water bath. Appearance of red or violet colour indicated the presence of protein.

9. Detection of carbohydrates

Molisch’s test: 2 ml of extract was treated with few drops of $\alpha$-naphthol solution. Formation of violet ring at the junction was indicated the presence of carbohydrate.

Fehling’s test: 2 ml of extract was hydrolysed with HCl, neutralized with NaOH and heated with Fehling’s solution A and B, formation of red precipitate indicated the presence of reducing sugar.

10. Detection of glycosides

Keller-killani test: 20 mg of plant extract was treated with 2 ml of glacial acetic acid containing few drops of FeCl$_3$. A brown coloured ring at the junction of two layers on adding 1-2 drops of concentrate H$_2$SO$_4$ was indicated the presence of glycosides.

11. Detection of amino acids

Ninhydrin test: To the two ml extract, 2 ml of Ninhydrin reagent was added and boil for few minutes. Formation of blue colour was indicated the presence of amino acids.

12. Detection of anthocyanin

In the two ml of aqueous extract, 2 ml of 2N HCl and then NH$_3$ was added. The change of colour from pink-red to blue-violet was indicated the presence of anthocyanin.
13. Detection of coumarin
On adding 3ml of 10% NaOH in 2ml of the aqueous extract, formation of yellow colour indicated the presence of coumarin in plant extract (Sawant and Godhate, 2013).

14. Detection of emodins
2ml of NH₄OH and 3ml of benzene was added to the extract, apperance of red colour was indicated the presence of emodins.

15. Detection of diterpene
Copper acetate test: Plant extract was dissolved in distilled water and treated with 10 drops of copper acetate solution, the formation of emerald green colour indicated the presence of diterpenes.

16. Detection of phytosterol
Salkowski test: 20 mg of extract was dissolved in chloroform which was treated with concentrated sulphuric acid and shaken well and allow standing. Appearance of golden red colour indicated the presence of phytosterol.

17. Detection anthroquinone
5 ml of extract was hydrolysed with dilute sulphuric acid, then 1ml of benzene and 1 ml of NH₃ was added. Formation of rose pink colour indicated the presence of anthroquinone in the plant extract.

18. Detection of Phlobatannins
On boiling the aqueous plant extract with 1% aqueous HCl, deposition of red precipitate indicated the presence of phlobatannins.

19. Detection of leucoanthocyanin
On adding 5 ml of isoamyl alcohol into 5ml of aqueous extract, appearance of red colour upper layer indicated the presence of leucoanthocyanin.

20. Detection of chalconese
On adding 2ml of NH₄OH to 0.5 gm ethanolic extract, apperance of red colour showed the presence of chalconese

Results and Discussion
The results of the phytochemical study of methanolic and ethanolic rhizome extract of C. longa have been presented in Table 1

<table>
<thead>
<tr>
<th>S.No</th>
<th>Extracting Solvents</th>
<th>Methanol</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Zn test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Protein</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Amino acids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Phenol</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Cardiac glycosides: Keller-Killant test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Phytosterol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12.</td>
<td>Anthocyanin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13.</td>
<td>Coumarin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>14.</td>
<td>Emodins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15.</td>
<td>Diterpene</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16.</td>
<td>Leucoanthocyanin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17.</td>
<td>Anthroquinone</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>18.</td>
<td>Chalcones</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19.</td>
<td>Phlobatannin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>20.</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Note: + = present, – = Absent

The phytochemical constituents present in the methanolic extract were more in number than that of ethanolic extract which may due to more extracting capacity of some specific phytochemicals like phenol, phlobatannin etc. In the study, phytochemical constituents alkaloids, steroids, tannins, saponins, flavonoids, phenol, carbohydrates, cardiac glycosides, phytosterol, protein, anthocyanin, coumarin, emodins, diterpenes, amino acids, phlobatannin, leucoanthocyanin, anthroquinone, chalcones, terpenoids were analysed. In the methanolic extract saponin, protein and amino acids, coumarin and phlobatannin were absent. In the ehanolic extract phenol, phlobatannin, protein, amino acids, coumarin and diterpenes were absent. The observed result was almost equivalent with the qualitative phytochemical screening of rhizomes of Curcuma longa linn which has been conducted by Sawant and Godhate (2013). Yellow coloring matter curcumins, other curcuminoids and essential oils were major bioactive ingredients showing various biological activities of turmeric. Some important pharmaceutical properties of turmeric were stated by Sabale et al., (2013). These properties were antiviral (Kim et al. 2001), antifungal (Tushar et al., 2010), anticancer, antidepresent, antimalarial and antimicrobial (Mishra et al. 2009), antiallergic (Tewtrakul et al. 2007), antiseptic (Banerjee and Nigam, 1978), antioxidiant (Braga et al., 2003), antiplatelet, (Deodhar et al., 1980), anti-inflammatory (Kohli et al., 2005), Lipid-lowering (Aktar and Taria, 2012) etc.

Conclusions

Plants are the major source of drugs in the modern as well as traditional system of medicine throughout the world. Curcuma longa has been in used as medicine from the time immemorial to treat wide range of ailments. Curcuma longa is an important medicinal plant that has a number of bioactive compounds. The phytochemical analysis of methanolic extract of C. longa showed the presence of some important phytochemicals like alkaloids, steroids, tannins, flavonoids, phenol, carbohydrates, cardiac glycosides, phytosterol, anthocyanin, coumarin, emodins, diterpenes, phlobatannin, leucoanthocyanin, chalcones, terpenoids. In the ethanolic extract except phenol, phlobatannin, coumarin and diterpenes the other phytochemicals which were observed in methanolic extract were also present. The previous phytochemical study of C. longa showed that it possesses antioxidant, anti-allergic, microbial, antibacterial, antiulcer, antivenum, antiemetic, antidepressant etc. properties. The study encourages the traditional system of medicine and will be applicable for the medicinal plant producer, research institutes, Ayurvedic institution and pharmaceutical companies for the manufacture of new drugs for treatment of various diseases. Further phytochemical analysis of C. longa using solvents of different polarity is essential for highlighting its more phytochemical constituents and medical properties.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the Head, University Department of Chemistry, Babasab Bhimrao Ambedkar Bihar University, Muzaffarpur, India for providing laboratory facilities and research environment. I would like to offer my gratitude to Prof. Dr. H.C. Rai former Head, University Department of Chemistry, B. R. A. Bihar University, India and Prof. Dr. C. B. Thapa, former Campus Chief, Prithvi Narayan Campus Pokhara, Nepal for providing research guidance, essential advice, valuable suggestions and inspiration during the research work.
REFERENCES


