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Comparative phytochemical analysis of *Curcuma longa* L. and *Curcuma caesia* Roxb., collected from Kandhamal district of Odisha, India

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ABSTRACT

The present study focuses on comparative phytochemical analysis *Curcuma longa* L. and *Curcuma caesia* Roxb. as both the species have a great medicinal value. The phytochemical analysis of *Curcuma longa* L. revealed presence of alkaloid, glycoside, saponin & carbohydrate and analysis of *Curcuma caesia* Roxb. showed the presence of alkaloid, phenolic compound, flavonoid, saponin, glycoside & carbohydrate. The GC-MS analysis of methanolic extract of *Curcuma longa* L. and *Curcuma caesia* Roxb. was carried out. Bioactive compounds like 9-Tricosene (Z), 1-Undescene & 1-Heptadecene, found in *Curcuma longa* L. have high medicinal properties and bioactive compounds like octanol and oleic acid are the reason behind anti-microbial and anti-inflammatory activity of *Curcuma caesia* Roxb. Functional groups like N-H, O-H, C-≡C, C=O, C=C, C-H, C-F, C-O-C, =C-H, C-O, C≡N, =C-H are present in both turmeric extracts, which can be proved useful in medicinal purpose.

1. Introduction

Turmeric, a plant of family ginger, commonly used as a spice, is also known for its medicinal value. It is the rhizome of the *Curcuma longa* L., belonging to the family Zingiberaceae. For cultivation of *Curcuma longa* L., requires 20^0-30^0 C temperature and a high rainfall (Arulmozhi *et al.,* 2006, Gul *et al.,* 2015, Sasikumar, 2005). This plant is native to South Asian countries and cultivated in India, China, Srilanka, Taiwan, Pakistan, Bangladesh, Thailand and Australia. More than 200 species of Curcuma are found in all over the world out of which 40-50 species are found in India.

Another most valuable species of Curcuma is *Curcuma caesia* Roxb. It is called as Black Turmeric. This species looks like *Curcuma longa* L. but the rhizomes are quite smaller than the rhizomes of *Curcuma longa* L. It is native to Nepal & North-East India, also grown in a few locations in central India. Due to the bluish black hue of its rhizome, it is also known as black turmeric having high economic importance (Devi *et al.,* 2015 and Pandey *et al.,* 2022) reported that bioactive compounds like carotenoids, flavonoids, saponins, tannin, phenolics, terpenoids etc., possess antioxidant, anticancer, anti-asthmatic, anxiolytic, antibacterial, antifungal & antimutagenic properties.

Plants are extremely important for conventional medicine. Turmeric has long been used to treat respiratory infections, wounds, burns, gastrointestinal and liver disorders. The phytochemicals present in plants like alkaloids, flavonoids, terpenoids, steroids, carotenoids play a provital role in pharmaceutical industries. A dimeric derivative of ferulic acid is curcumin. The main bioactive component of turmeric powder is curcumin. Additionally, it

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has choleretic, anti-inflammatory, antiseptic, antibacterial and carminative effects. Now-a-days curcumin is widely used as food additives with coloring, flavoring and preservative properties. Curcumin content of black turmeric is higher than yellow turmeric. Black turmeric contains 14.8% curcumin and yellow turmeric contain 6.3% (Bohra *et al.,* 2021) which can be used not only for scientific research but also in the manufacturing of food and other products.

Pakkirisamy *et al.,* in 2017 conducted the GC-MS, FT-IR, and phytochemical analysis of the methanolic extract of black turmeric *Curcuma caesia* Roxb., and found the presence of tanins, alkaloids, terpenoids, flavonoids, phenol and saponin. Fifteen compounds were found by phytochemicals and chromatography, and functional groups such N-H, O-H, C=C, and CH_3 are also present (Momoh *et al.,* 2022) also in GC-MS analysis of bioactive compounds present in *Curcuma longa* L. rhizome extract (Gaikwad *et al.,* 2022). In view of this, the present study looks at potential phytochemicals found in both turmeric with pharmaceutical importance.

2. Materials and Methods

Collection Site: Odisha's Kandhamal district is famous for its production of natural growing turmeric, which has more healing properties than other turmeric. Kandhamal turmeric earned GI (Geographical Indication) tag from Intellectual property India on 1st April 2019. Kandhamal district is present in the central part of Odisha. This district lies between 19^0-34^0 N to 20^0-36^0 North latitude and 83^0-34^0 East to 84^0-34^0 East longitude.

Collection of Plant Materials: The rhizomes of yellow turmeric (*Curcuma longa* L.) and black turmeric (*Curcuma caesia* Roxb.) were collected from Dharampur, a village of Kandhamal district of Odisha, during the month of December and January because at this time the rhizomes of both turmeric are grown perfectly and cultivated. Then, the rhizomes were packed in paper packets and collected for experimental purpose. The yellow turmeric rhizomes were packed in paper packets having named 'T' and the black turmeric rhizomes were packed in paper packets having name 'BT.' The rhizomes were washed thoroughly so that the soil particles are removed from the rhizomes. Then the extract of both rhizomes was extracted.

Preparation of Methanolic Extract: For the preparation of extract, outer skin of rhizomes was removed and washed thoroughly. Then the rhizomes were chopped into pieces and crushed with mortar pestle. Then they were mixed with 80% methanol. Then the extract of each turmeric was transferred to several volumetric flasks, kept in orbital shakers for one week. Then the extracts of each turmeric were filtered using filter paper. Rotary evaporation was performed on the evaporate all the remaining methanol.

Figure 1 : Showing Rhizomes of *Curcuma caesia* Roxb. & *Curcuma longa* L.

Phytochemical Screening for Turmeric Extract: Two test tubes containing extracts of *Curcuma longa* L. and *Curcuma caesia* Roxb. was taken in 100 ml conical flasks. Then the conical flasks were marked as 'T' for normal turmeric & 'BT' for black turmeric. Then the test for phytochemical screening is carried out.

Test for Alkaloids: After being dissolved in 3 ml of diluted HCl, the extract was filtered out of the mixture. The filtrate underwent the following alkaloid test.

A) DRAGENDROFF'S TEST: A few drops of Dragendroff's reagent were added to the 1ml filtrate. Presence of Alkaloids will be proved by the reddish-brown precipitate.

B) MAYER'S TEST: Two drops of Mayer's reagent were added to 1ml of the filtrate in a test tube. Occurrence of white or creamy precipitate will indicate the test tube as positive.

C) WAGNER'S TEST: To 1ml of extracts in a test tube, two drops of Wagner's reagent were applied. Reddish-brown precipitate will indicate a successful test.

Test for Phenolic Compunds and Tanins:

A) Ferric Chloride test: A few drops of a neutral 5% ferric chloride solution were added after the 1ml of extract had been dissolved in the 5ml of D.W. The presence of phenolic chemical will be indicated by the coloration being dark green.

B) Gelatin's test: 5ml of D.W. and 2ml of a 1% solution of gelatine containing 10% NaCl were added after 1ml of the extract had been dissolved. The presence of phenolic compounds will be revealed by the appearance of white precipitate.

Test for Flavonoids: Alkaline reagent test: 10% ammonium hydroxide solution was used to treat 1 millilitre of the extract. The presence of flavonoids will be confirmed by the formation of yellow fluorescence.

Test for Glycosides: Borntrager's test: In a test tube, 1ml of extract was heated with 1ml of H_2SO_4 for 5 minutes and then filtered while still hot. Following cooling, the filtrate was shaken with an equivalent volume of dichloromethane or chloroform. Dichloromethane or chloroform was divided into layers, and the lowest layer was shaken with half of its volume of diluted ammonia. The ammonial layer will form a rose pink to crimson hue.

Test for Saponin: 3ml of extracts was diluted with D.W & volume was made up to 10 ml. The suspension is shaken vigorously in a graduated cylinder for 15min. Occurrence of a 2cm layer of foam will indicate the presence of saponins.

Test for Carbohydrates:

A) Molisch's test: A few drops of naphthol solution should be added to 1ml of aqueous extract. Shake the test tube and slowly add conc. H_2SO_4 from the side wall. The presence of carbohydrate in the extract will be confirmed by the formation of a red, violet ring at the intersection of two liquids.

B) Fehling's test: 10ml of 50% HCl was mixed into 2ml of the extract in a test tube. Then the mixture was heated in a water both for 30 min. Add 5ml of Fehling's solution & the mixture was boiled for 5 min. Presence of Glycosides will be visible as a brick-red precipitate.

C) Benedict's Test: 2ml of aq. Extract, add few drops of benedict's reagent and heat. Green, yellow, or red colour shows the presence of carbohydrates.

Test for Proteins and Amino Acids:

Biuret's test: In 1.5ml of aq. Extract, add 1.5ml biuret's reagent in test tube for 30 minutes. A violet colour produced will show the present of proteins.

Fourier Transform Infrared Spectrophotometer (Ftir) Analysis: Fourier transform infrared spectrophotometer (FTIR) analysis is the most powerful tools for identifying the types of chemical bonds or functional groups present in extracts. The methanolic extracts of both the turmeric extracts were used for the FTIR analysis.

GC-MS Analysis: The GC-MS test was performed by Central Instrumental Faculty, OUAT, Bhubaneswar. Methanolic extracts were investigated through Gas Chromatography Mass Spectrometry/Mass Spectrometry Electron Ionization (GC-MS/EI) mode. The GC-MS is a Perkin Elmer Clarus 590 model.

3. Result

Phytochemical Screening: The screening for phytochemicals of methanolic extract of both the turmeric was performed (Raaman, 2006).

Test for Alkaloid:

- Dragendroff's test: Reddish brown precipitate was appeared in both test tubes
- Mayer' test: Test tube carrying black turmeric extract showed white or creamy precipitate but in the test tube carrying normal turmeric extract showed slightly less creamy precipitate.
- Wagner's test: Both test tubes showed reddish brown precipitate.

Test for Phenolic Compound:

- Ferric chloride test: Dark green colour was not appeared in both test tubes.
- Gelatin test: Test tube containing black turmeric showed slightly white precipitate but test tube containing normal turmeric did not showed any precipitate.

Test for Flvonoid:

- Alkaline reagent test: Yellow fluorescence is appeared in test tube containing black turmeric extract but not appeared in normal turmeric extract.

Test for Glycoside:

- Borntrager's test: A rose-pink colour is appeared in test tube containing black turmeric extract and light pink colour is appeared in test tube containing normal turmeric extract.

Test for Saponin:

- A 2cm layered foam is appeared in both the test tubes.

Test for Carbohydrates:

- Molisch's test: A red violet ring formed between the junctions of two liquids in both the test tubes.
- Benedict's test: Both the test tubes did not show any green, red, and yellow colour.
- Fehling's test: Test tube containing black turmeric extract showed slightly brick-red precipitate but test tube containing normal turmeric extract did not show any precipitate.

Test for Protiens and Amino Acids:

- Biuret's test: Both the test tubes did not show violet colour.

GC-MS Analysis:

The bioactive substance found in black turmeric and turmeric's methanolic extract is by GC-MS analysis is showed in following tables. Both the turmeric extracts contain very useful bioactive compounds. They can be used as production of various medicines.

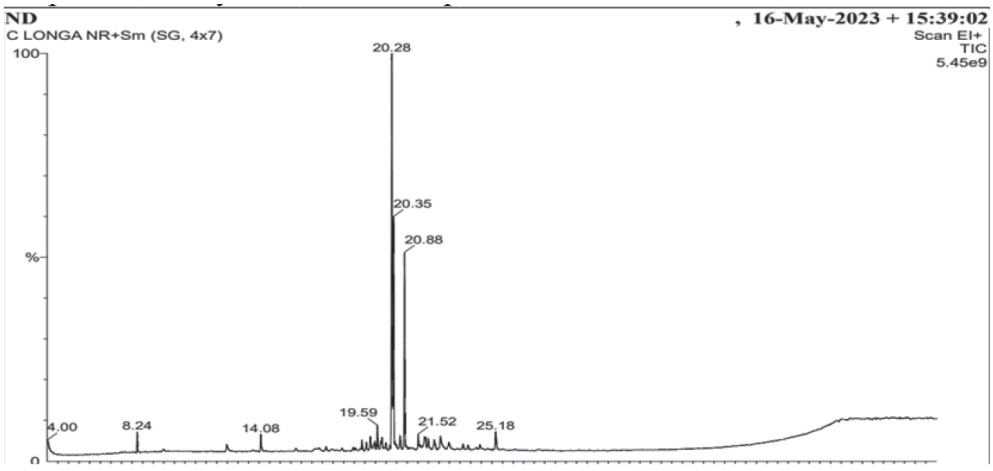

Figure 2: Showing GC-MS Analysis of *Curcuma longa* L.

Table 1:

Bioactive Compounds Found in *Curcuma longa* L.

COMPOUND NAME	MOLECULAR WEIGHT	MOLECULAR FORMULA
5-METHYL-Z-5-DOCOSENE	322	$C_{23}H_{46}$
11-TRICOSENE	322	$C_{23}H_{46}$
9-TRICOSENE, (Z)-	322	$C_{23}H_{46}$
TRIFLUOROACETIC ACID, PENTADECYL ESTER	324	$C_{17}H_{31}F_3O_2$
PENTAFLUOROPROPIONIC ACID, PENTADECYL ESTER	374	$C_{18}H_{31}F_5O_2$
CIS-1-CHLORO-9-OCTADECENE	286	$C_{18}H_{35}Cl$
9-EICOSENE, (E)-	280	$C_{20}H_{40}$
CYCLOUNDECANE, (1-METHYLETHYL)-	196	$C_{11}H_{22}$
5-EICOSENE, (E)-	280	$C_{20}H_{40}$
1-HEPTADECENE	238	$C_{17}H_{34}$
9-NONADECENE	266	$C_{19}H_{38}$
E-15-HEPTADECENAL	252	$C_{17}H_{32}O$
1-UNDECENE, 5-METHYL-	168	$C_{11}H_{22}$
E-14-HEXADECENAL	238	$C_{16}H_{30}O$
1-OCTADECENE	252	$C_{18}H_{36}$
CETENE	224	$C_{16}H_{32}$
1,2-CYCLOHEXANEDIOL, 3-METHYL-6-(1-METHYLETHYL)-, (1.ALPHA.,2.BETA.,3.BET	172	$C_6H_{12}O_2$
9-OCTADECENE, (E)-	252	$C_{10}H_{20}O_2$
ACETIC ACID, CHLORO-, OCTADECYL ESTER	346	$C_{27}H_{45}ClO_3$
1-HENEICOSYL FORMATE	340	$C_{22}H_{44}O_2$

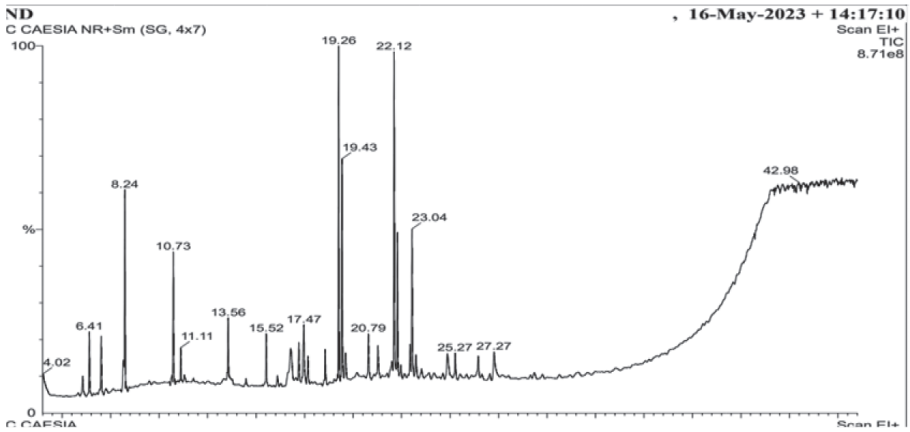

Figure 2 : Showing GC-MS Analysis of *Curcuma caesia* Roxb.

Table 2:

Bioactive Compounds Found in *Curcuma caesia* Roxb.

COMPOUND NAME	MOLECULAR WEIGHT	MOLECULAR FORMULA
2H-PYRAN, 2,2'-[1,10-DECANEDIYLBIS(OXY)] BIS [TETRAHYDRO-	342	$C_{20}H_{38}O_4$
OCTANAL	128	$C_8H_{16}O$
CHLOROMETHYL 2-CHLOROUNDECANOATE	268	$C_{12}H_{22}Cl_2O_2$
Z-8-METHYL-9-TETRADECENOIC ACID	240	$C_{15}H_{28}O_2$
2-N-HEXYLTHIOLANE, S, S-DIOXIDE	204	$C_{10}H_{20}O_2S$
1-DECANOL, 10-[(TETRAHYDRO-2H-PYRAN-2-YL) OXY]-	258	$C_{15}H_{30}O_3$
4-METHYLTHIANE, S, S-DIOXIDE	148	$C_7H_{14}O_2S$
2-ETHYLTHIOLANE, S, S-DIOXIDE	148	$C_6H_{12}O_2S$
2H-PYRAN, TETRAHYDRO-2-(12-PENTADECYNYLOXY)-	308	$C_{20}H_{36}O_2$
2-PIPERIDINONE, N-[4-BROMO-N-BUTYL]-	233	$C_9H_{16}BrNO$
OLEIC ACID	282	$C_{18}H_{34}O_2$
3-N-HEXYLTHIOLANE, S, S-DIOXIDE	204	$C_{10}H_{20}O_2S$
UNDEC-10-YNOIC ACID, 4-METHYL-2-PENTYL ESTER	266	$C_{17}H_{30}O_2$
CYCLOHEXANOL, 2,3-DIMETHYL-	128	$C_8H_{16}O$
BUTYL 9-TETRADECENOATE	282	$C_{18}H_{34}O_2$
CYCLOHEXANOL, 3,3-DIMETHYL-	128	$C_8H_{16}O$
4-METHYL-Z-4-HEXADECEN-1-OL	254	$C_{17}H_{34}O$
BUTYL 9-HEXADECENOATE	310	$C_{20}H_{38}O_2$
4-N-HEXYLTHIANE, S, S-DIOXIDE	218	$C_{11}H_{22}O_2S$
5,5-DIMETHYL-CYCLOHEX-3-EN-1-OL	126	$C_8H_{14}O$

Discussion

The present work aims at novel bioactive compounds from the extracts of rhizomes of indigenous plants *Curcuma longa* L. and *Curcuma caesia* Roxb. Both the turmeric extract and its phytochemical examination revealed that their rhizomes are rich in phytochemicals. The alkaloid tests showed that alkaloid present in both the normal turmeric and black turmeric. The Gelatine test showed the presence of phenolic compounds in black turmeric, which is absent in yellow turmeric. Flavonoid is also present in extract of black turmeric and absent in yellow turmeric. Glycosides present in both the turmeric extracts. Carbohydrate was found to be present in the black turmeric but absent in yellow turmeric. Protein and amino acid test also showed negative response to both turmeric extracts. The presence of highly beneficial bioactive chemicals on the methanolic extracts of *Curcuma longa* L. and *Curcuma caesia* Roxb. was discovered by GC-MS analysis. 9-Tricosene (z) which is found in *Curcuma longa* L. is used as pesticide and 1-Heptadecene is used as making scent and used as cosmetic industries and 1-Undescene, 5-methyl is used for making medicines. The bioactive compounds which are found in *Curcuma caesia* Roxb. are also very useful like Octanol is used in perfume and flavour production and Oleic acid is used as components in many food materials and used as an excipient in pharmaceuticals. Both the turmeric extracts contain useful bioactive compounds which can be used for making various products.

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