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Chemical analysis and antibacterial activity of Black turmeric (*Curcuma caesia* Roxb.) genotypes

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Abstract
Curcuma caesia Roxb. is an important, lesser known, non-conventional medicinal plant belonging to Zingiberaceae family. The eight genotypes selected were GKM-1, GKM-2, GKB-3, GKB-4, GKJ-5, GMV-6, GKT-29 and GKK-30 were collected from different regions as presented in (Table 1). The Rhizomes of black turmeric genotypes were extracted with methanol at room temperature for 18 h. Preliminary phytochemical analysis revealed the presence of tannins, flavonoid and phenol. The highest phenol GMV-6 (1.46 $mg^{-1}g$), tannin GKT-29 (0.69 $mg^{-1}g$) and flavonoid GMV-6 (1.64 $mg^{-1}g$) was recorded. The bioactive compounds of *Curcuma caesia* Roxb have been evaluated using GC-MS. Totally 19 major compounds were identified from eight genotypes. The major constituents were identified in the methanolic rhizome extracts were Curcumenol in GKM-1(20.35%), GKB-3(12.96%), GKB-4(15.67%) and in GKT-29(16.17%), Curcumenone in GKM-1(16.07%), GKM-2(25.03%), GKB-3(29.30%) and in GKT-29(22.25%), Epicurzerenone in GKM-1(12.93%) and GKM-2(21.82%), 9-12, Octadecadionic acid in GKM-1(5.54%), GKB-3(2.81%) and in GKB-4(3.59%), 2-Bornanone in GKM-2(4.38%) and in GKB-3(5.38%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- in GKB-3(8.91%), GKB-4(24.44%), GKJ-5(19.82%) and GKT-29(9.88%), Isoborneol in GKM-2(1.54%) and GKB-3(3.67%) and Eucalyptol in GKM-2(1.17%). The research findings have shown that the rhizome of *C. caesia* is extensively rich in secondary metabolites. The extracts were assessed for their potential antibacterial activity against Gram positive and Gram negative bacteria *viz.*, *Bacillus cereus*, *Bacillus subtilis*, *Streptococcus agalactiae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella flexneri*. The rhizome extracts of black turmeric genotypes exhibit positive results against the Gram positive bacteria.

Keywords: Black turmeric, genotypes, chemical properties and antibacterial activity

Introduction
India has long history of using plants for medicinal purposes as mention in Ayurveda**.** The significance of medicinal plants for prevention, mitigation and cure of diseases are always recognized. History revealed that plants have been a valuable source of natural products for maintaining human health at all the times. Their importance is continuously growing now days. Most of the people now prefer natural therapies to get rid of from serious side effects of some of the present day medication.

Curcuma caesia Roxb. is an important, lesser known, non-conventional medicinal plant belonging to Zingiberaceae family and genus C*urcuma*. It is commonly called as black turmeric. *Curcuma* is one of the biggest genera in the Zingiberaceae family and contains around 80 species (Larsen, 2005) [14]. It is native to North-East and central India, distributed to Java, Myanmar and rarely found in Madhya Pradesh, Jharkhand, Chhattisgarh, Orissa and other parts of South India. This species is commonly found along with the coastal areas and river alluvial soils extending up to midlands of Kerala and South Karnataka in wild form (Sabu, 2006) [23]. It grows well in moist deciduous forest area. The rhizomes of the plant are aromatic in nature. The inner part of the rhizome is bluish-black in colour and emits a characteristic sweet smell, due to presence of essential oil. Traditionally, the rhizomes of *Curcuma caesia* Roxb. Are used in treating many diseases like piles, asthma, cancer, leprosy, wounds, impotency, fertility, tooth ache, leukoderma, allergies, vomiting, tumors, tuberculous glands of the neck, enlargement of the spleen, epileptic, rheumatic arthritis, paste of fresh rhizome is applied during snake and scorpion bite, used as tonic for the brain and the heart (Dewangan *et al.,* 2014) [6]. *Curcuma caesia* Roxb. has been categorized as endangered due to great demand, indiscriminate exploitation and limited cultivation (Neha *et al.*,2014) [18]. The analytical technique GC-MS was used to identify the compounds.

The present study was carried out to identify the chemical compounds present in it. The antimicrobial activities of this plant have not yet been explored. In this context the study was carried out to screen the antibacterial efficacy of *Curcuma caesia* against both Gram positive and Gram negative bacteria *viz*., *Bacillus cereus, Bacillus subtilis, Streptococcus agalactiae, Escherichia coli, Pseudomonas aeruginosa* and *Shigella flexneri*.

Material and Methods

The biochemical profiling was done at Quality analytical laboratory, Department of Horticulture, UAS, GKVK, Bengaluru. The matured rhizomes were harvested, cleaned, cut into small pieces, was shade dried for 20 days and ground in to powder. Known quantities of the ground rhizome material were extracted with methanol using soxhlet apparatus for 18 hrs at constant temperature of 72 ^{0}C (Jyothi and Rajeshwari, 2012 [13]; Dutta, 2015) [9]. The amount of total phenolic content, total flavonoid content and total tannin content was determined according to standard methods. (Donald *et al*., 2001; Sarangathem and Haokip, 2010) [7, 24].

The GC-MS analysis was done at Biofuel department, UAS, GKVK, Bengaluru. The methanolic rhizome extract was analysed using Shimadzu QP2020 series gas-chromatograph using, SH-Rtx Wax 30 m x 0.50 µm x 0.25 µm Dia column. Helium was used as the carrier gas at a flow rate of 1.5 ml min^{-1} at constant pressure. Injection volume was 1 µL and a spilt ratio of 1:100 was used. The pressure was maintained at 35.6 kPa. Detection was done with a flame ionization detector at 240 ºC. The oven program was as follows, set point 50 ºC was held for 1 minutes and then increased to 220 ºC at 10 ºC min^{-1} and held at 240 ºC for 3 min at 5 º C min^{-1}. All samples were analyzed and values were reported. The mass spectrum of the sample was identified by computer comparison against a mass spectral library.

The antibacterial activity was done at microbiology laboratory, Department of microbiology UAS, GKVK Bengaluru. The methanolic rhizomes extracts of black turmeric (*Curcuma caesia* Roxb.) was tested for their antibacterial activity towards the following human pathogenic bacterial cultures *Bacillus cereus* (MCC 2236), *Bacillus subtilis* (MCC 2183), *Streptococcus agalactiae* (MCC 3039), *Escherichia coli* (MCC 2552), *Pseudomonas aeruginosa* (MCC 3097) and *Shigella flexneri* (MCC 3095) collected from National Centre for Microbial Resource (NCMR), Pune, India. The antibacterial activity was determined by the agar well diffusion assay. Full strength agar plates were prepared and were seeded with the pathogenic bacteria using a sterile spreader. A well of 2 mm dia was borne at the centre of the agar plate. The methanol is used as negative control. 100 µl of methanolic rhizome extract was introduced in to the well at a concentration of 50 mg/ml and methanol as negative control. The plates were incubated at 30 °C for 24 hours and were observed for the formation of halo zones around the wells. The diameter of the zones was measured using scale.

Table 1: Treatment details

Treatments/Genotypes	Region of collection	State
T_1 (GKM-1)	Mijar	Karnataka
T_2 (GKM-2)	Mangalore	Karnataka
T_3 (GKB-3)	Bengaluru	Karnataka
T_4 (GKB-4)	Sanjeevini vatika Dept. of Horticulture, UASB	Karnataka
T_5 (GKJ-5)	Joida	Karnataka
T_6 (GMV-6)	Vidarbha-gadehirolli	Maharashtra
T_7 (GKT-29)	Thrissur-vellanikara	Kerala
T_8 (GKK-30)	IISR Kozhikode	Kerala

Results and Discussion

Plants are very important source of potentiality useful bioactive principles for the development of new chemotherapeutic agents. In the present study, the exploration of phytochemical screening with methanol extract of *Curcuma caesia* revealed the presence of total phenol, total tannin and total flavonoid compounds which are known to have remedial activity against diseases producing pathogen. Therefore, it can be used pharmacologically to develop new compounds for health benefit (Table 2).

The highest total phenolic compounds (1.46 mg/g) were recorded in genotype GMV-6, followed by genotypes GKB-4 (1.19 mg/g) and GKB-3 (1.13 mg/g). Whereas, genotype GKM-1 (0.21 mg/g) recorded least total phenolic compounds. Genotype GKT-29 had the highest tannin content (0.69 mg/g), followed by genotypes GKJ-5 (0.53 mg/g) and GKK-30 (0.45 mg/g). While the lowest tannins (0.18 mg/g) were found in genotype GKB-3. Genotype GMV-6 had recorded highest flavonoid (1.64 mg/g), followed by genotypes GKB-4 (1.14 mg/g) and GKK-30 (1.02 mg/g). While lowest flavonoid (0.05 mg/g) was found in genotype GKT-29. The variation in the content of phenols, tannins and flavonoids was due to role and effect of microclimate. Other factors may influence the variation were soil conditions *viz.*, texture, nutrient status, management practices, maturity of the rhizome and part of the rhizome used for extraction etc., The bioactive compounds vary within the species as well as from place to place owing to different agro climatic conditions and environment. The results of quantitative analysis of black turmeric rhizomes for phenols, tannins and flavanoids were in accordance with the studies of Sarangthem and Haokip (2010) [24]; Jyothi and Rajeshwari (2012) [13]; Baghel *et al.* (2013) [2]; Neha *et al.* (2014) [18]; Nawi *et al.* (2014) [16]; Jose and Thomas (2014) [12]; Dutta (2015); Pakkiriswamy *et al.* (2017) [22]; Ranemma and Reddy (2017) [22]; Nayak and Bhatnagar (2019) [17] and Borah *et al.* (2020) [4].

Table 2: Phytochemical compounds present in different black turmeric genotypes

Genotypes	Total Phenol Content (TPC) (mg/g)	Total Tannin Content (TTC) (mg/g)	Total Flavonoid Content (TFC) (mg/g)
GKM-1	0.21	0.19	0.20
GKM-2	0.54	0.26	0.62
GKB-3	1.13	0.18	0.51
GKB-4	1.19	0.22	1.14
GKJ-5	0.49	0.53	0.84
GMV-6	1.46	0.36	1.64
GKT-29	0.29	0.69	0.05
GKK-30	0.83	0.45	1.02
S.Em±	0.05	0.02	0.06
F-test	*	*	*
CD@5%	0.14	0.06	0.18

GC-MS analysis revealed wide variation in the chemical composition of rhizome extract of *Curcuma caesia* Roxb genotypes (Table 3). The genotypes originated from Karnataka and Arunachal Pradesh are deficient in camphor Borah *et al.* (2020) [4]. Genotypes GKM-1 (20.35%), GKB-3 (12.96%), GKB-4 (15.67%) and GKT-29 (16.17%) rhizome extracts had appreciable amount of sesqueterpene compound curcumenol.

Curcumenone was the major bioactive component of GKM-1 (16.07%), GKM-2 (25.03%), GKB-3(29.30%) and GKT-29 (22.25%). The palmitic acid or hexadeconoic acid was found in genotype GKM-1 (13.69%). The higher percentage of sesqueterpene *viz.*, epicurzerenone was observed in rhizome extract of genotypes GKM-1 (12.93%) and GKM-2 (21.82%).

9,12-Octadecadionic acid were the major component of rhizome extracts GKM-1 (5.54%), GKB-3 (2.81%) and GKB-4 (3.59%). Higher percentage of 5-hydrocxy methyl furfural was found in genotype GKB-4 (15.05%). The terpinoid *viz.*, 2-Bornanone was major component in genotypes GKM-2 (4.38%) and GKB-3 (5.38%).

l-(+)-Ascorbic acid 2,6- dihexadecanoate was the major component in genotype GKM-2 (2.63%). Genotypes GKB-3 (8.91%), GKB-4 (24.44%), GKJ-5 (19.82%) and GKT-29 (9.88%) had 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- as a major constituent of rhizome.

The terpinoid *viz.*, isoborneol was found major constituent in the rhizome extracts of GKM-2 (1.54%) and GKB-3 (3.67%). The higher percentage of eucalyptol was found in genotype GKM-2 (1.17%). 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- was the major component of genotype GKJ-5 (16.57%).

GKM-1 (1.03%), GKM-2 (1.23%), GKB-4 (1.34%) and GMV-6 (1.10%) had α-Terpineol as major constituent of the rhizome. Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)- were the major constituents of the rhizome extract of GKB-3 (1.18%), GMV-6 (2.34%) and GKK-30 (0.68%). Considerable quantity of Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans- was found in genotype GKK-30 (1.09%).

Butanoic acid, 2-methyl-3-oxo-, ethyl ester was the major component found in genotype GKJ-5 (4.10%). 3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene), (E,E) were the major components of GKM-1 (2.18%) and GMV-6 (2.33%). 9,12-Octadecadienoic acid (Z,Z)- was identified at higher percentage in GKM-1 (5.54%), GKM-2 (2.17%), GKB-4 (3.50%) and GKJ-5 (2.09%) rhizome extract.

These results are in line with the findings of Hikino *et al.* (1975) [11]; Dosoky *et al.* (2019) [8] Pandey and Chowdhary (2003) [20]; Mukunthan *et al.* (2014) [15]; Borah *et al.* (2020) [4] and Fatt *et al.* **(**2021) [10]. The volatile compounds detected in rhizome extract of *Curcuma caesia* genotypes were summarized in to sesquiterpenes, monoterpenes and trace amount of phenolic groups. Bioactive constituents of black turmeric are dependent on its geographical distribution.

Besides, work carried out by Behuria and Srivastava (2004) [3] and Borah *et al.* (2020) [4] provides a strong base to further explore and repurpose rhizome extract based on geographical collection. The absence of camphor in our report gives us an apparent depiction that the rhizomes grown in Karnataka are deficient of camphor.

Table 3: Major bioactive compounds found in rhizome extract of black turmeric genotypes

Sl. No	Compound	Genotypes with per cent of compounds
1	Curcumenol	GKM-1(20.35%), GKB-3(12.96%), GKB-4(15.67%), GKT-29(16.17%)
2	Curcumenone	GKM-1(16.07%), GKM-2(25.03%), GKB-3(29.30%), GKT-29(22.25%)
3	n-hexadecanoic acid	GKM-1(13.69%)
4	Epicurzerenone	GKM-1(12.93%), GKM-2(21.82%)
5	9-12,Octadecadionic acid	GKM-1(5.54%), GKB-3(2.81%), GKB-4(3.59%)
6	5-hydrocxy methyl furfural	GKB-4 (15.05%)
7	2-Bornanone	GKM-2(4.38%), GKB-3(5.38%)
8	l-(+)-Ascorbic acid 2,6- dihexadecanoate	GKM-2(2.63%)
9	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	GKB-3(8.91%), GKB-4(24.44%), GKJ-5(19.82%), GKT-29(9.88%)
10	Isoborneol	GKM-2(1.54%), GKB-3(3.67%)
11	Eucalyptol	GKM-2(1.17%)
12	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	GKJ-5(16.57%)
13	α-Terpineol	GKM-1(1.03%),GKM-2(1.23%), GKB-4(1.34%), GMV-6(1.10%)
14	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	GKB-3(1.18%), GMV-6(2.34%), GKK-30 (0.68%)

15	Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans-	GKK-30(1.09%)
16	Butanoic acid, 2-methyl-3-oxo-, ethyl ester	GKJ-5(4.10%)
17	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethylidene), (E,E)	GKM-1(2.18%), GMV-6(2.33%)
18	9,12-Octadecadienoic acid (Z,Z)-	GKM-1(5.54%), GKB-4(3.50%), GKJ-5(2.09%), GKM-2(2.17%), GKB-4(3.50%)
19	Oleic acid	GKM-1 (20.35%), GKM-2(8.83%) GKM-3(12.96%), GKB-4(1567%), GKT-29 (16.17%)

The study was undertaken to examine the antibacterial activity of promising black turmeric genotypes against six selected human pathogens. The result revealed that the highest zone of inhibition against the *Bacillus cereus* was seen in the methanolic rhizome extract of genotype GKM-2 (12.16 mm), followed by GKJ-5 (8.12 mm), GMV-6 (7.96 mm) and least was observed in GKK-30 (6.18 mm). Against the *Bacillus subtilis*, the genotype GKB-4 (13.81 mm) had shown maximum zone of inhibition, followed by GKM-1 (13.20 mm), GMV-6 (12.14 mm) and least was observed in methanoilc rhizome extract of GKJ-5 (6.21 mm). The methanolic rhizome extract of GKT-29 (7.23 mm) exhibited maximum zone of inhibition against the *Streptococcus agalctiae*, followed by the genotypes GMV-6 (6.82 mm) and GKJ-5 (6.78 mm) and least was observed in methanoilc rhizome extract of GKB-3 (3.23 mm). Similar results were recorded by Chowdhary *et al.* (2010) and Pandey and Gupta (2014) [21].

All the genotypes could control only Gram positive pathogens. The higher resistance of Gram negative pathogens against the rhizome extract is due to thick murein layer in their outer membrane, acts as barrier for entry of inhibitory substance in to the bacterial cell and also Gram negative bacteria having outer phospholipid membrane carrying the structural lipopolysaccharides components, which makes the cell wall impermeable to secondary metabolites present in extract. The Gram positive has single layered cell wall with the peptidoglycan constituting the outer layer (Pandey and Gupta, 2014) [21].

The antibacterial activity of individual rhizome extract not only depends on the biochemical constituents of the genotypes, it also reflects the varied concentration, culture efficacy *etc.*, and the variation of the antibacterial activities of various extracts can be explained by various influencing factors such as polarity of the solvents used, polarity of the compounds extracted by various solvents, in addition to the diffusion coefficient of the solvent in the media used in assay (Anjana *et al.*, 2009) [1].

Table 4: Antibacterial activity of different black turmeric genotypes

Genotypes	T_1	T_2	T_3	T_4	T_5	T_6
	Zone of inhibition (mm)					
GKM-1	6.32 (±0.09)	13.20 (±0.12)	4.92 (±0.09)	-	-	-
GKM-2	12.16 (±0.12)	8.13 (±0.12)	5.18 (±0.10)	-	-	-
GKB-3	7.13 (±0.10)	7.23 (±0.10)	3.23 (±0.09)	-	-	-
GKB-4	7.52 (±0.10)	13.81 (±0.12)	5.49 (±0.10)	-	-	-
GKJ-5	8.12 (±0.12)	6.21 (±0.09)	6.78 (±0.12)	-	-	-
GMV-6	7.96 (±0.12)	12.14 (±0.12)	6.82 (±0.12)	-	-	-
GKT-29	6.41 (±0.09)	6.52 (±0.09)	7.23 (±0.12)	-	-	-
GKK-30	6.18 (±0.09)	7.12 (±0.10)	6.12 (±0.10)	-	-	-

Conclusion

The present work has been performed to establish the various Phyto-chemical and GC-MS parameters, which could serve as important and has commercial interest in both research institutes and pharmaceutical companies for the manufacturing of the innovative drugs. This primary information will facilitate in conducting further studies on discovery of bioactive constituents, resolve of their efficacy by *in vivo* studies and demonstration of their safety and efficacy in clinical trials. The Results revealed that the rhizome extracts could control only Gram positive bacteria. The above findings reveal that the plant based antimicrobials have enormous therapeutic potentials and can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials.

References

1. Anjana PM, Bindhu MR, Umadevi M, Rakhi RB. Antibacterial and electrochemical activities of silver, gold and palladium nanoparticles dispersed amorphous carbon composities. Appl. Surf. Sci. 2009;479:96-104.
2. Baghel S, Baghel R, Sharma K, Sikarwar I. Pharmacological activities of Curcuma caesia. Int. J Green Pharm. 2013;7:1-5.
3. Behuria S, Srivastava VK. Essential oils of leaves of *Curcuma* species. J. Essen. Oil Res. 2004;16:109-110.
4. Borah S, Priyanka S, Hemanta Kumar S. Chemical profiling, free radical scavenging and anti-acetyl cholinesterase activities of essential oil from *Curcuma caesia* of Arunachal Pradesh, India. Pharmaco. Res. 2020;12(1):76-84.
5. Chowdhury Z, Islam MS, Hoque MR, Chowdhury AMA, Bhuiyan RH, Alauddin M. Antibacterial and antifungal activity of ethanol extract of *Curcuma casiea*. Chittagong Univ. J. Biol. Sci, 2010, 149-154.
6. Dewangan MK, Dwivedi C, Sivna PL, Rao SP, Yadav R, Chandrakar K, *et al*. Medicinal value of *Curcuma caesia* Roxb: An overview. Earth Journals. 2014;3(4):1-9.
7. Donald MCS, Prenzler PD, Autolich M, Robarts K. Phenolic content and antioxidant activity of olive extracts. Food Chem. 2001;73:73-84.
8. Dosoky NS, Noura S, Satyal P, Setzer WN. Variations in the volatile compositions of *Curcuma* species, Foods. 2019;28(53):15-18.
9. Dutta B. Study of secondary metabolite constituents and curcumin contents of six different species of genus *Curcuma*. J. Med. Plants Stud. 2015;3(5):116-119.
10. Fatt LC, Rahman NWA, Aziz MAA, Isa KM. Identification of the chemical constituents of *Curcuma caesia* (black turmeric) hydrosol extracted by hydro-distillation method. IOP Conf. Ser. Earth Environ. Sci. 2021;765(1):1-8.
11. Hikino H, Konno C, Agatsuma K, Takemoto T, Horibe I, Tori K, *et al*. Sesquiterpenoids. Part XLVII. Structure, configuration, conformation, and thermal rearrangement of furanodienone, isofuranodienone, curzerenone, epicurzerenone, and pyrocurzerenone, sesquiterpenoids

of *Curcuma zedoaria*. J Chem. Soc. 1975;1(5):478-484.
12. Jose S, Thomas TD. Comparative phytochemical and anti-bacterial studies of two indigenous medicinal plants *Curcuma caesia* Roxb. and *Curcuma aeruginosa* Roxb. Int. J Green Pharm. 2014;8(1):29-32.
13. Jyoti S, Rajeshwari S. Evaluation of phytochemical constituent in conventional and non-conventional species of Curcuma. Int. Res. J Pharm. 2012;3(8):203-204.
14. Larsen K. Distribution patterns and diversity centres of Zingiberaceae in SE Asia. Biologiske Skrifter. 2005;55:219-228.
15. Mukunthan KS, Anil Kumar NV, Balaji S, Trupti NP. Analysis of essential oil constituents in rhizome of *Curcuma caesia* Roxb. from south India. J Essen. Oil Bear. Pl. 2014;17(4):647-651.
16. Nawi NM, Simoh S, Zainal A, Rahman Z. Proceeding of the 30th annual seminar of "The Malaysian Natural Products Society". Int. Conf. Nat. Prod, 2014, 60-66.
17. Nayak S, Bhatnagar S. Antioxidant, cytotoxic and phytochemical assessment of rhizomes of black turmeric (*Curcuma caesia*). Int. J Agri. Inno. and Res. 2019;7(3):366-369.
18. Neha B, Tiwari KL, Jadhav SK. A review on non-conventional turmeric *Curcuma caesia* Roxb. Curr. Trend. Biotech. Pharm. 2014;8(1):91-101.
19. Pakkirisamy M, Suresh Kumar K, Karthikeyen R. Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of *Curcuma caesia* Roxb (black turmeric), Pharmacogn J. 2017;9(6):952-956.
20. Pandey AK, Chowdhary AR. Volatile constituents of rhizome oil of *Curcuma caesia* Roxb. from central India. *Flavour*. Frag. J. 2003;18:463-465.
21. Pandey D, Gupta AK. Antibacterial efficacy of *Curcuma caesia* from Bastar district of Chhattisgarh, India. Int. J Pharm. Sci. Res. 2014;5(6):2294.
22. Ranemma M, Reddy KS. Phytochemical investigation study of *Curcuma caesia* Roxb. different geographical regions (Delhi and Orissa) of India. J Biotech. and Biochem. 2017;3(1):23-26.
23. Sabu M. Zingiberaceae and Costaceae of South India. Kerala: *Indian Asso. Angio. taxo.*, Dep. Bot. Calicut Uni., Kerala, India, 2006, 132-138.
24. Sarangthem K, Haokip MJ. Secondary metabolites of *Curcuma* species. Int. J Appl. Agric. Sci., 2010.
25. Sasikumar B. Genetic resource of Curcuma: diversity, characterization and utilization. Plant Genetic Resource. 2005;3:230-251.