# Phytochemical screening and FT-IR analysis of different crude extracts of *Curculigo orchioides* G

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## ABSTRACT

*Curculigo orchioides* Gaertn. is a rare rasayana herb (family Amaryllidaceae) popularly known as "Kali Musli". Traditionally used in Ayurvedic medicine as an aphrodisiac and adaptogen, the plant is native to india. The medicinal plant commonly found in Asian countries such as India, Japan, China, and Nepal. is a rhizomatous monsoon perennial with plicate leaves flowering in July-August, occurs in forest floors of moist deciduous forests as well as in open lateritic rocky plateaus amongst grasses. It is commonly known as Kali Musli an endangered medicinal plant and need to be conserved and domesticated. In recent times, there is has been upsurge of interest in focus on the importance of medicinal plants and traditional health systems in solving the health care problems of the world. It is widely used as a medicinal plant, and *Curculingo orchioides* rhizome extracts contain an alkaloid lycorine, sterols including sitosterol, sapogenin, and flavones glycoside 5,7- dimethoxy glucopyranoside which are used in the treatment of asthma and jaundice. Including impotency, aphrodisiacs, tonics, and skin conditions restorative; used in filarial, fever, diarrhea. There are many scientists who have investigated its antioxidant, anticancer, and hepatoprotective properties. The phytochemical analysis of *Curculigo orchioides* parts of the tubers are used to obtain several phytochemical constituents. They are like that alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins, steroids and phenolic compounds. In my study, i found four extracts for screening analysis, Methanol, and chloroform, n- hexane, acetone: the methanolic extract found in Alkaloid, Flavonoid, Glycoside, Steroids, Phenols, Tannins, and Terpenoids; the chloroform extract found in Alkaloid, Glycoside, Steroids, and Terpenoids; and the acetone extract found in Alkaloid Flavonoid, Steroids, Phenols, and Terpenoids; the n- hexane extract found in Alkaloids, flavonoid, steroids, and terpenoids. The research aimed at phytochemical screening and FT-IR analysis of methanol, chloroform, acetone, and n- hexane extract of *Curculigo orchioides.*

**Keywords**: Curculigo orchioides, seasonal plant, phytochemical screening, FT-IR Analysis

## INTRODUCTION

Curculingo orchioides gaerth , is one of the well known medicinal plant belonging to the family hypoxidaceae [1,2]. it is distributed widely in the southern parts of japan, china, india, and Australia, generally used as a tonic in traditional Chinese medicine to treat decline in physical strength [3,4,5]. The plant commonly reffered to as golden eye grass, nilappanai, kali musli etc [6,7]. is a rhizomatous erect perennial herb of medicinal importance and a native of india [8,9]. leaves are sessile or shortly petiolate; leaf blade lanceolate to linear usually 10-45 x 0.5-2.5 cm, laxly pilose or glabrous, base tapering, apex narrowly acuminate with prominent veins [10,11]. The flower are yellow [12,13]. This is a mansoon plant, which rows for a period of three months, particularly, November to January and then disappears on completion of its life cycle [14,15,]. The plants of the genus curculingo contain diverse secondary metabolites wirh a varity of bio-actives [16,17]. Up to now 10 species including C.orchioides, C.capitulata, C. sinensis, C. crassifolia, C. breviscapa, C.gracilis, C. recurvata, C. glabrescens, C. pilosa, and C. latifolia have been chemically and pharmacologically investigated [18,19]. Medicinally, it is used for treatment of piles, jaundice, asthma, diarrhea and on pimples this plants has now become endangred due to depletion in the natural habitate as it has been harvested by local people with low income.urinary tract infections (UTI) are infections that affect millions of people worldwide [20,21]. Gram negative bacteria are the major causative agents of about 90% of urinary tract infections while rest of the cases includes gram positive bacteria [22,23]. The effectiveness of drugs was declined gradually due to the emergence of multidrug resistant pathogens resulted from over use of antimicrobial medicines [24,25]. One of the highly useful plants in the indigenous system of medicine is black musale (C. orchioides) pharmacological studies showed that C. orchioides are used in several disorders such as adaptogenetic, anti- inflammatory, anticonvulsant, sedative, androgenic and immune- promoting activities [26,27,28]. It helps to overcome erectile impotence by relaxing the smooth muscles of corpora cavernosa, the electile tissue wereby more blood can be pumped into it [29,30]. The plant flavonone glycoside is a powerful uterine stimulant in guinea pigs, rats and rabbits [31,32]. C. orchioidedes is an acaluesecent herb found in the sub- tropical Himalayas from kumanu eastwards to khasia hill, Manipur, bihar, chota Nagpur, west Bengal and ghats, the root tuber is used in siddha system for the treatment of diseases like diabetes , leucoderma, pain and as an aphrodisiac [33]. They are revealed that phenols and phenolic glucoside, norlignans, and terpenoids were the main constituents in the curculigo species these compounds and extracts of curculigo plants exhibited various bio-activities including adaptive immunomodulation radical scavenging and anti-oxidation, sweet- tasting and taste- modifying, anti- inflammation aphrodisiac, estrogeneic and sexual behavior- modifying, neuroprotection, antidepression, nephroprotection, anti-osteoporotic, antiarthritic, antitumor, mast cell stabilization and antihistaminic, and antidiabetic activity [34]. The isolation, structurs and bio- activities of the compounds from species belonging to genus curculigo, have been previously reviewed [35,36]. These monographs presented the traditional use, phytochemistry, pharmacology and toxicology of plants in the

genus curculigo, however, the phytochemicals of the medicinal plants of genus curculigo are inadequately reviewed and the related pharmacological advances are not updated [37].

## MATERIALS AND METHODS

PREPRATION OF PLANT EXTRACT

Fresh tubers were collected and, washed thoroughly under running tap water, washed with sterile distilled water, and cut in to the tuber's in slender shapes under the shade. They were grind in to coarse powder by using a mechanical pulverizer. About 100g of powder were repeatedly extracted with e methanol, acetone, n- hexane, and ethyl chloroform in a 500 ml round bottom flask with 250 ml of solvent. The reflux time for each solvent was 20 cycles for complete extraction for using the Soxhlet apparatus. The extract was filtered and finally dried at room temperature. the filtrate was collected and concentrated by using a rotary evaporator (Heidolph ) under controlled conditions of temperature and pressure.

**Phytochemical Screening**

Chemical tests for the screening and identification of bioactive chemical constituents like alkaloids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, proteins, amino acids, flavonoids, and tannins, in the medicinal plants under study were carried out in extracts using standard procedures.

1. ***Screening for Alkaloids***

   Crude extract was mixed with 2 ml of 1% HCL and heated gently. mayer and wagner reagents were then added to the mixture, and the turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2. ***Screening for Flavonoids***

   Crude extract was mixed with a few fragments of magnesium ribbon, and concentrated HCL was added drop – wise. A pink scarlet color appeared after a few minutes, which indicated the presence of flavonoid.

3. ***Screening for Glycosides***

   Crude extract was mixed with 2 ml of chloroform, then 2 ml of concentrated $H_2SO_4$ was added carefully and shaken gently. A reddish brown color indicated the presence of a steroidal ring ,i.e glycone protein of the glycoside.

### *4. Screening for Saponins*

Crude extract was mixed with 5 ml of distilled water in a test tube, and it was shaken vigorously. The formation of stable forms was taken as an indication of the presence of saponins.

### *5. Test for Steroids*

Crude extract was mixed with 2 ml of chloroform, and concentrated $H_2SO_4$ was added sideways. A red color was produced in the lower chloroform layer, as indicated .the presence of steroids another test was performed by mixing crude extract with 2 ml of chloroform and then 2 ml of each of concentrated $H_2SO_4$ and acetic acid were poured into the mixture. The development of greenish coloration was indicated . the presence of steroids.

### *6. Screening for phenols*

Crude extract was mixed with 2 ml of a 2% solution of $Fecl_3$ a blue - green or black coloration indicated the presence of phenols .

### *7. Screening for Tannins*

Crude extract was mixed with 2 ml of a 2% solution of $Fecl_3$ a blue - green or black coloration indicated the presence of tannins.

### *8.* Screen for carbohydrates

To 3 ml of test solution in a test tube 2-3 drops of freshly prepared molisch reagent (20% α-naphthol solution in 95% ethanol) was added. Heating may be required. Then 2ml of concentrated sulphuric acid was added. Heating may be required. Then 2 ml of concentrated sulphuric acid was added slowly along the wall of the test tube .formation of a purple/ violet ring at the junction of two layers indicates the presence of carbohydrates

### *9. Screening for Terpenoids*

Crude extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this, 2 ml of concentrated $H_2SO_4$ was added and heated for about 2 minutes. a grayish color indicated the presence of terpenoid.

**Table 1: preliminary phytochemical tests for the presence of active constituents in *C. orchioides* tuber extract**

| S.NO | Solvent's extract/ name of the Compound's | n-hexane | chloroform | Acetone | Methanol |
|---|---|---|---|---|---|
| **1.** | Glycosides | _ | + | + | +++ |
| **2.** | Phenols | + | ++ | ++ | +++ |
| **3.** | Alkaloids | + | _ | + | ++ |
| **4.** | Flavanoids | + | + | _ | ++ |
| **5.** | Saponins | + | _ | + | +++ |
| **6.** | Steroids | + | _ | _ | +++ |
| **7.** | Carbohydrates | + | ++ | _ | + |
| **8.** | Terpenoids | + | + | + | + |
| **9.** | Tannins | + | ++ | _ | + |

(+ = Slight; ++ = Normal; +++ = High; - = Negative)

## FT-IR ANALYSIS

The range of Infrared region is 12800 ~ 10 $cm^{-1}$ and can be divided into near-infrared region (12800 ~ 4000 $cm^{-1}$), mid-infrared region (4000 ~ 200 $cm^{-1}$) and far-infrared region (50 ~ 1000 $cm^{-1}$). The discovery of infrared light can be dated back to the 19th century. Since then, scientists have established various ways to utilize infrared light. Infrared absorption spectroscopy is the method which scientists use to determine the structures of molecules with the molecules' characteristic absorption of infrared radiation. Infrared spectrum is molecular vibrational spectrum. When exposed to infrared radiation, sample molecules selectively absorb radiation of specific wavelengths which causes the change of dipole moment of sample molecules. Consequently, the vibrational energy levels of sample molecules transfer from ground state to excited state. The frequency of the absorption peak is determined by the vibrational energy gap. The number of absorption peaks is related to the number of vibrational freedom of the molecule. The intensity of absorption peaks is related to the change of dipole moment and the possibility of the transition of energy levels. Therefore, by analyzing the infrared spectrum, one can readily

obtain abundant structure information of a molecule. Most molecules are infrared active except for several homonuclear diatomic molecules such as $O_2$, $N_2$ and $Cl_2$ due to the zero dipole change in the vibration and rotation of these molecules. What makes infrared absorption spectroscopy even more useful is the fact that it is capable to analyze all gas, liquid and solid samples. The common used region for infrared absorption spectroscopy is 4000 ~ 400 $cm^{-1}$ because the absorption radiation of most organic compounds and inorganic ions is within this region.

FTIR spectrometers are the third generation infrared spectrometer. FTIR spectrometers have several prominent advantages (1) The signal-to-noise ratio of spectrum is significantly higher than the previous generation infrared spectrometers. (2) The accuracy of wavenumber is high. The error is within the range of ± 0.01 cm . (3) The scan time of all frequencies is short (approximately 1 s). (4) The resolution is extremely high (0.1 ~ 0.005 cm ). (5) The scan range is wide (1000 ~ 10 cm ). (6) The interference from stray light is reduced. Due to these advantages, FTIR Spectrometers have replaced dispersive IR spectrometers.

**Figure: 1 FT-IR analysis of N-hexane tuber extract of *C.orchioides***

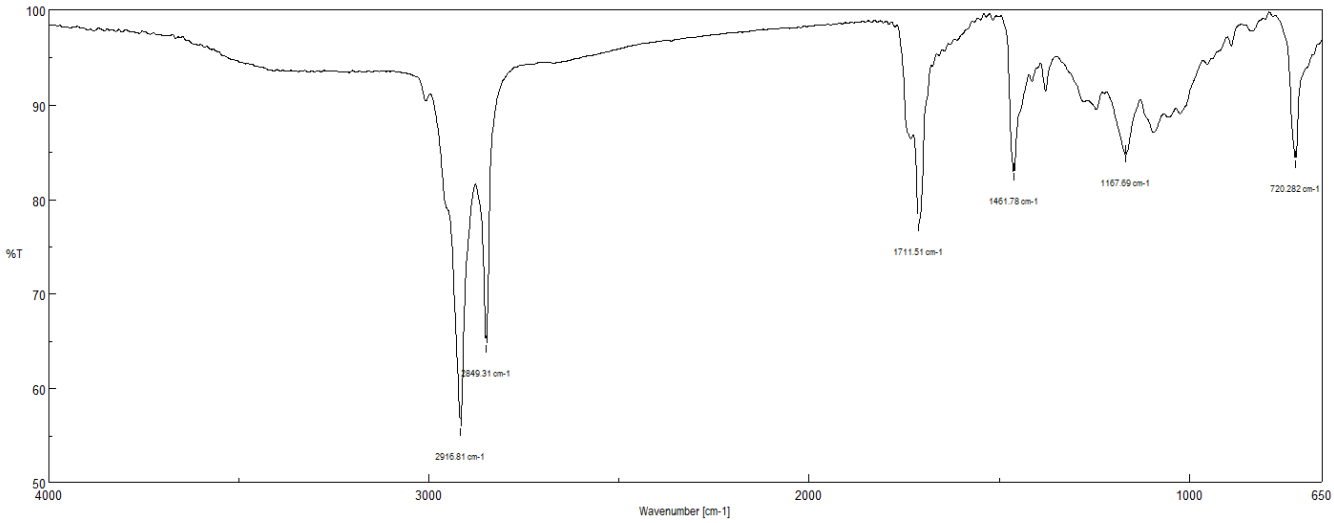


**Table 2: FT-IR peak values with functional group of *C.orchioides* N-hexane Tuber extract.**

| S.NO | Frequency $cm^{-1}$ | Absorption intensity | Vibrating system | Band assignment | Type of compounds |
|---|---|---|---|---|---|
| 1. | 2916.81 | Medium | Stretching | C-H | Alkane |
| 2. | 2849.31 | Medium | Stretching | C-C | Alkane |
| 3. | 1711.51 | Strong | Stretching | C=O | Carboxylic acid |
| 4. | 1461.78 | Medium | Bending | C-H | Alkane |
| 5. | 1167.69 | Strong | Stretching | S=O | Sulfonamide |
| 6. | 720.282 | Strong | Bending | C=C | Alkene |

**Figure: 2 FT-IR analysis of Acetone tuber extract of *C.orchioides***

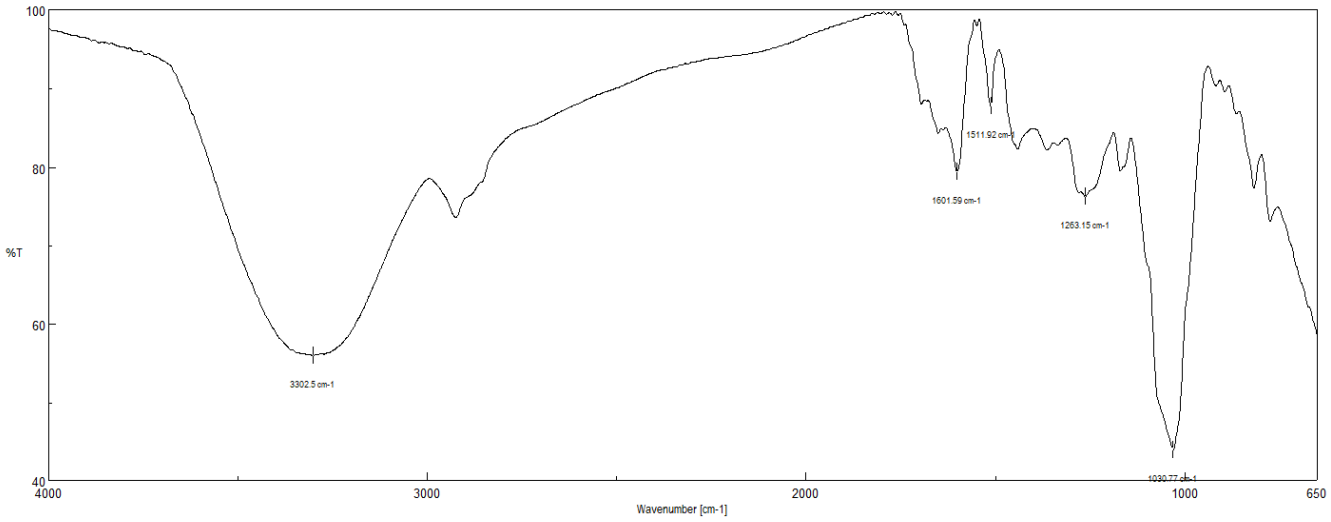


**Table 3: FT-IR peak values with functional group of *C.orchioides* Acetone Tuber extract.**

| S.NO | Frequency $cm^{-1}$ | Absorption intensity | Vibrating system | Band assignment | Type of compounds |
|---|---|---|---|---|---|
| 1. | 3302.5 | Medium | Stretching | N-H | Aliphatic primary amine |
| 2. | 1601.59 | Medium | Stretching | C=C | Conjugated alkene |
| 3. | 1511.92 | Strong | Stretching | N-O | Nitro compound |
| 4. | 1263.15 | Strong | Stretching | C-O | Aromatic ester |
| 5. | 1030.77 | Strong | Stretching | CO-O-CO | Anhydride |

**Figure: 3 FT-IR analysis of Chloroform tuber extract of *C.orchioides***

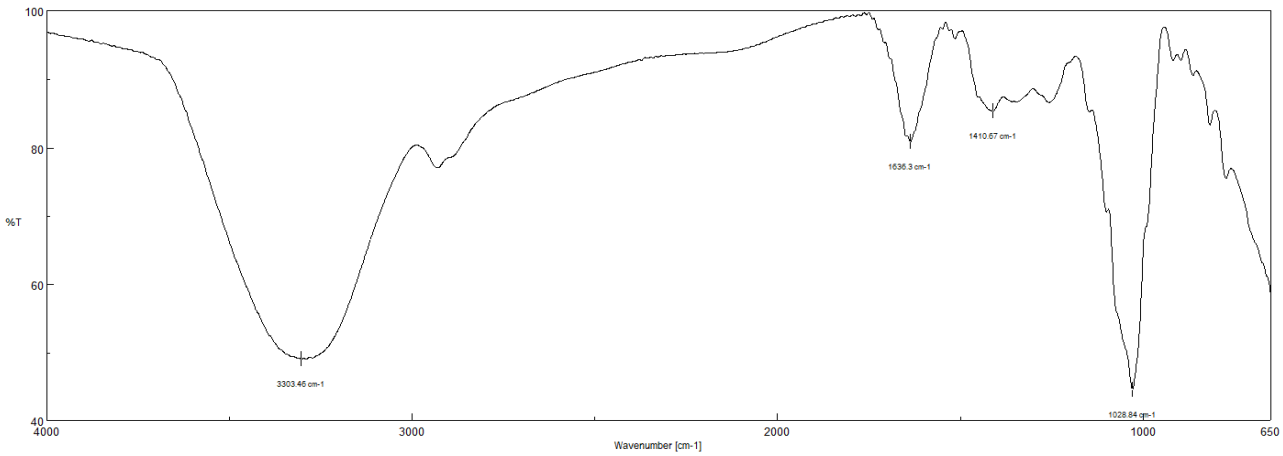

**Table 4: FT-IR peak values with functional group of *C.orchioides* Chloroform Tuber extract.**

| S.NO | Frequency $cm^{-1}$ | Absorption intensity | Vibrating system | Band assignment | Type of compounds |
|---|---|---|---|---|---|
| 1. | 3303.46 | Medium | Stretching | N-H | Secondary amine |
| 2. | 1636.3 | Strong | Stretching | C=C | Alkene |
| 3. | 1410.67 | Strong | Stretching | S=O | Sulfonyl chloride |
| 4. | 1028.84 | Strong | Stretching | S=O | Sulfoxide |

**Figure: 4 FT-IR analysis of Methanol tuber extract of *C.orchioides.***

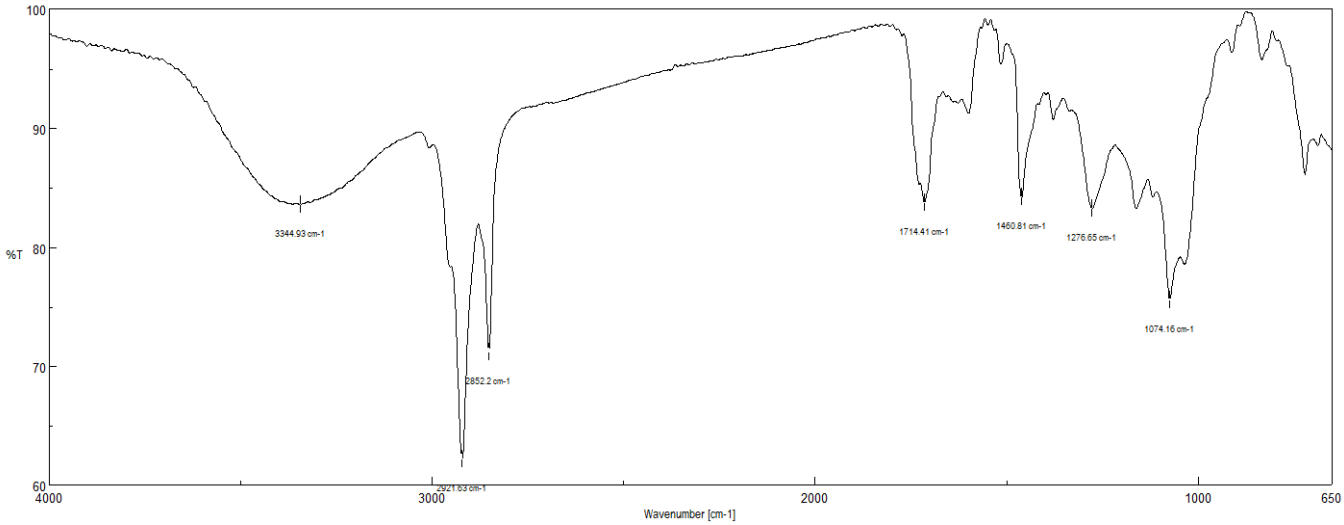


**Table 5: FT-IR peak values with functional group of *C.orchioides* Methanol Tuber extract.**

| S.NO | Frequency $cm^{-1}$ | Absorption intensity | Vibrating system | Band assignment | Type of compounds |
|---|---|---|---|---|---|
| 1. | 3344.93 | Medium | Stretching | N-H | Secondary amine |
| 2. | 2921.63 | Medium | Stretching | C-H | Alkane |
| 3. | 2852.2 | Medium | Stretching | C-H | Alkane |
| 4. | 1714.41 | Strong | Stretching | C=O | Carboxylic acid |
| 5. | 1450.81 | Medium | Bending | C-H | Alkane |
| 6. | 1276.65 | Strong | Stretching | C-O | Aromatic ester |
| 7. | 1074.16 | Strong | Stretching | C-O | Primary Alcohol |

## RESULT AND DISCUSSION

The bioactive chemicals examined in the tuber extract of C.orchioides revealed the presence, absence, and not detection of compounds as listed Ethanol, methanol, acetone, and extracts of Curculigo orchioides tubers contain alkaloids, flavonoids, glycosides, saponins, steroids, tannins, and terpenoids, with an exeption emphasis on phenols. According to the stated statement that the ethanol, methanol, acetone, and extract of C.orchioides contains at least all the phytochemicals except phenolic compounds. The FT-IR spectrum of tuber extract (prepared in different solvents)of Curculigo orchioides are given in the data on the peak value and the probable functional groups obtained by (FT-IR) analisis present in the tuber extract (prepared in N-hexane, Chloroform, Acetone, Methanol) Plants contain various components that have often been used to make natural medicines. Because of the unparalleled richness of chemical compounds, plant-derived natural products, as a standardized extract or in pure form, give limitless prospects for new therapeutic leads. The present study used FTIR to identify the functional groups in extracts of C. orchioides post preliminary phytochemical analysis. The peak value and the functional group are displayed in (Table-2,3,4,5). The characteristic absorption bands were exhibited at 2916.81cm−1 , 2849.31 cm−1 , 1711.51cm−1 , 1461.78 cm−1 , 1167.69 cm−1 ,720.282 cm−1 , Table -2 3302.5 cm−1 , 1601.59 cm−1 , 1511.92 cm−1 ,1263.15 cm−1 , 1030.77 cm−1 , Table-3 3303.46 cm−1, 1636.3 $cm^{-1}$, 1410.67 $cm^{-1}$, 1028.84 $cm^{-1}$ Table -4 3344.93 $cm^{-1}$, 2921.63 $cm^{-1}$, 2852.2 $cm^{-1}$, 1714.41 $cm^{-1}$, 1450.81 $cm^{-1}$, 1276.65 $cm^{-1}$, 1074.16 $cm^{-1}$, Table-5 2920.55 $cm^{-1}$, 2851.24 $cm^{-1}$, 1713.44 $cm^{-1}$, 1457.92$cm^{-1}$, 1378.85 $cm^{-1}$,1158.04 $cm^{-1}$, 720.28 $cm^{-1}$. The presence of the C-H stretch at 2916.81 cm−1 indicates the presence of alkane in the extract. The characteristic stretching band of N-H was observed at 3302.5 cm−1 , 3303.46 cm−1 and 3344.93 cm−1 indicating the presence of amine group. In agreement, the ethanolic leaf extracts of C. orchioides showed a broad absorption spectrum of N-H stretch at the region of 3344.93 cm−1 , indicating the presence of the amine group. Additionally, O-H bending vibrations were observed in the fingerprint region, indicating the presence of an phenolic functional group similar to our findings. These studies indicate that the. extract is rich in chemical constituents of alkaloids, phenolic, and saponins; implicating their attributes to the reported bioactivity [38,39,40]. are presented in Plants are very important source of potentially useful bioactive principles for the development of new chemotherapeutic agents [41,42]. The biological and pharmacological properties of many plants are still unknown [43,44]. World over, the scientists are exploring the potential of utilizing pharmacologically active compounds from medicinal plants [45]. 11 Herbal medicines are used by 80% of the people worldwide due to its high efficiency, cheap cost, nonnarcotic nature and fewer side effects [46]. 12 In the present study, the exploration of phytochemical screening with Methanol extract of Curcuma caesia revealed the presence of carbohydrate, flavonoid, steroid, phenol, alkaloid, tannin, amino acid, terpenoid and glycoside compounds which are known to have remedial activity against diseases producing pathogen [47]. Therefore it can be used pharmacologically to develop new compounds for health benefit Phytochemical constitutes of plants serves as defense mechanism against by many microorganisms [48]. The therapeutic properties of medicinal plants are possibly due to the

presence of various secondary metabolites [49]. 13 Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and improvement [50].

## CONCLUSION

Medicinal plants are natural sources of bioactive compounds to treat life threatening diseases. C.orchioides is an important medicinal plant, used as an antidote for skin diseases, is in demand commercially. This tuber when consumed in high quantities. This plant is also considered a Chlorophenolic glucosides, B,C source for the chemical constituents of the medicine industry. Additionally, it would be useful to produce a high amount of Chlorophenolic glucosides, B,C for Anti-osteoporosis based on natural products. Several studies have reported that C.orchioides is rich in various biologically active compounds, which could serve as a potential source of crude drugs that can be used as a complementary source of traditional medicines.

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