Profiling bioactive compounds in the bark of *Cinchona officinalis* L. and anti-arthritic properties thereof

ABSTRACT

Thebark samples of *C. officinalis*were collected from The Nilgiris district of Tamil Nadu. The collected samples were processed and extracted using methanol solvent. The extracts showed high yield of secondary metabolites especially alkaloids, phenols, flavonoids, steroids, terpenoids, sterols, quinones and tannins. Alkaloid content was very high in *C.officinalis* followed by phenol, flavonoid, steroid and Tannin ~~contents~~.The secondary metabolites present in *C. officinalis* (Bark) were isolated, purified, identified and characterized using differential Chromatographic techniques such as TLC, HPLC and GC-MS/ MS. The compounds identified using High Performance Liquid Chromatography (HPLC) technique in bark of *C. officinalis*in the retention times of 1.750, 2.250, 4.016 and 7.266 minutes were quinine, isoquinidine and cinchonidine. The compounds were then characterized using GC- MS/ MS technique with reference to NIST library. The compounds were eluted from the bark of *C. officinalis* in the retention time of 9.569, 10.488, 11.814, 13.593, 14.282, 16.542, 16.967, 23.494, 24.954, 25.151, 25.988, 26.752, 26.982, 27.906, 28.277, 31.454, 33.604, and 35.744 minutes. By correlating the retention times with (NIST) mass spectral library the eluted compounds were confirmed as Phthalic acid, Hexadecanoic acid,1,2,3,5-Cyclohexanetetrol, Cinchonab-9-one, 9,12-Octadecadienoic acid (Z,Z)-, Heptadecanoic acid, Quinine, Cinchonine, Cinchonidine, Phenol, Quinine, and gamma.-Sitosterol. The phytochemicals present in the *C. officinalis* (Bark) samples were evaluated for their biological properties viz., anti-inflammatory activity and anti-arthritic activity in in-vitro conditions. Anti- inflammatory properties of plant samples were tested by protein denaturation method using diclofenac (75 mg/ ml concentration) as positive control. The methanol extract of *C.officinalis* (Bark) showed anti- inflammatory properties of range between 17.0 and 57.3%. Anti- arthritic activity of bark extract of *C. officinalis* (75 mg/ ml concentration) was evaluated by albumin denaturation method using diclofenac (75 mg/ ml concentration) as positive control. The methanol extract of *C.officinalis* (Bark) showed anti- arthritic properties of range between 18.4 and 58.5%. From the study it is understood that the alkaloids and polyphenols present in the methanolic extract of bark of *C. officinalis* were responsible for their biological properties. Hence, *C. officinalis* can serve as an alternative to synthetic drugs available in the market to treat arthritissubject to safety (toxicity) studies in humans. Based on the *Invitro* results, it was confirmed that the bark of *C.officinalis* possesses comparatively high biological values. Thus an herbal aerosol for arthritis may be formulated using the bark extract of *C.officinalis* as key ingredient and the therapeutic product will be of cost effective, easy to use and pocket friendly.

Keywords: Cinchona officinalis, arthritis, Phyto-compounds, HPLC, and GCMS

1.INTRODUCTION

Arthritis is the leading cause of disability in adults worldwide, limiting the daily activities for billions of people(Briggs, et al.,2016). Globally more than 100 types of arthritis are there. The most common arthritis are osteoarthritis, rheumatoid arthritis, and psoriatic arthritis, and septic arthritis, which are autoimmune diseases, often originate from the joints (Aiyalu et al., 2017). Arthritis affects people of all ages and has been known to mankind since ancient times. Scientists found evidence of arthritis in the knees of Nefertari's mummy. Mitch Leslie reports in science that the study of old knees provides evidence supporting this model and suggests that arthritis may not be the fate of old age.

The symptoms of arthritis may include joint pain, stiffness, tenderness, lack of movement, grating inside the joints, fever, chillsandredness. and heat occur in the joint can cause tissue damage. Ultimately, because of the avascular nature of cartilage, once damage has occurred, it cannot be repaired, thus making a cure essentially impossible (EI- Gabalawy *etal*., 2002). Treatment aims to control pain, reduce joint damage and improve or maintain quality of life. It includesphysical therapy, medication and patient education and support. Ancient medicine is also used to treat arthritis. Clinical studies conducted in recent years have helped shed light on this disease. Although there is no cure for arthritis, researchers have found many treatments to control symptoms. Treatment of arthritis depends on the nature and severity of the underlying disease. The main goal is to reduce pain and improve the function of affected joints before more serious problems occur.

NSAIDs are anti- inflammatory, analgesic, and antipyretic agents, and they are typically used to reduce chronic pain, decrease stiffness and improve function in patients with osteoarthritis, Rheumatoid arthritis and other forms of arthritis (Simon, 2013). In addition, steroidal drugs, Immunosuppressant's, Corticosteroid medicinesand synthetic drugssuch ashydroxychloroquine, leflunomide, methotrexate and sulfasalazinare often prescribed medicine for arthritis. Though the synthetic drugs are efficient in pain relief, long term intake of these drugs can cause wide range of side effects. Studies of older adults show that chronic NSAIDs use increases the risk of peptic ulcer disease, acute renal failure, and stroke/ myocardial infections (Marcum *et al*., 2010). Although research has not proven that specific herbs or supplements can treat arthritis, many herbs can reduce joint pain. There are so many herbal products available in the market for oral intake and topical application including tablets, powder, syrup, oil and spray etc. The existing topical formulations have the disadvantages like difficulty in applying the formulation to respective site, staining cloth due to greasy nature, more chances of contamination and more wastage due to inaccuracy of dose (Aiyalu *et al*., 2017).

Cinchona officinalis possess many biological properties which includesantimalarial(Permin et al., 2016), Lupus erythematosus(Lubov et al., 2022), SARS COVID 19(Arumugam et al., 2024) and arthritis. Considerably less number of research works is carried out to substantiate the efficacy of *C. officinalis*against arthritis. In this work the bark of *C. officinalis* was selected due to the potentially active biological ingredients present in it. The present study focused on the profiling active compounds in bark extract of *C. officinalis* and evaluation of anti-inflammatory and anti-arthritic activities thereof. The phytochemicals present in the bark extract of *C. officinalis* were qualitatively and quantitatively estimated using standard protocols followed by characterization using differential chromatographic techniques such as thin layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Gas Chromatography coupled with double Mass Spectrometer (GC-MS/ MS). Subsequently anti-inflammatory and anti-arthritic activities of *C. officinalis* (Bark)were also evaluated to determine its ant-arthritic efficacy.

2.MATERIALS AND METHODS

The bark samples of *C. officinalis* were collected from the original sources at Medicinal Plants Development Area (11º25'37" N Latitude and 76º43'48" E Longitude), Cinchona Village, Doddabetta, The Nilgiris district, Tamil Nadu (INDIA). Qualitative and quantitative evaluation of methanol extract of bark of cinchona was performed by following the standard protocols to detect and quantify the phytochemicals present in it (Harborne, 1984;Raaman, 2006; Rajesh et al., 2014). Plant metabolites like alkaloids, phenols, flavonoids and Tannins were isolated and characterised using differential chromatographic techniques such as TLC, HPLC and GC-MS/ MS (Senthilkumar et al., 2012). The biological properties like anti- inflammatory and anti- arthritic activites of the secondary metabolites were determined by Protein denaturation (Williams *et al*., 2008) and Albumin denaturation (Vaijanthimala *et al*., 2019) methods respectively.

3.RESULTS AND DISCUSSION

The bark of *C. officinalis* collected from The Nilgiris district of Tamil Nadu was authenticated by a taxonomist at Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Tamil Nadu. The percentage yield of bark of *C.officinalis* was found to be 71.1% using methanol as solvent.Plants and herbs are rich sources in phytochemicals that prevent/treat or reducemany health issues. Qualitative evaluation of phytochemicals in the methanol extract of bark sample *C.officinalis* revealed the presence of plant metabolites such as proteins, alkaloids, phenols, flavonoids, steroids, Terpenoids, Sterols and Quinones. Alkaloids such as colchicine, colchicoresin, demo-colchicine foundin *Colchicum autumnale* (Colchicum) (Sultana *et al*., 2014), ~~and~~ strychnine, brucine and brucine N-oxide in Strychnosnux-vomica (Nux-vomica) (Bhati*et al*., 2012),barbaloin~~a glycoside and an anthraquinone~~andAloe emodin in Aloevera were reported to have anti-inflammatory activity(Shelton, 1991; Kshirsagar*et al*., 2014).

Table 1. Qualitative and quantitative analysis of phytochemicals in methanol extract of *C.officinalis* (Bark)

S.No	Phytochemical constituents	Name of the conformation test	Phytochemical screening	Phytochemical quantificationMg/g
1	Alkaloids	Mayer's Test	+	**0.24**
2	Phenols	Lead acetate Test	+	**0.82**
3	Flavonoids	H_2SO_4 Test	+	**1.61**
4	Steroids	Salkowski Test	+	**2.86**
5	Terpenoids	Salkowski Test	+	**0.13**
7	Sterols	Salkowski Test	+	**22.79**
8	Quinones	H_2SO_4 Test	+	**33.40**

3.1.Thin Layer Chromatography Analysis

Phytochemicals such as alkaloids, phenols, flavonoids and tannins find major applications in therapeutics. Hence these compounds were isolated and characterized using differential chromatographic techniques.

Table 2:Retention factor of alkaloids, phenols, flavonoids and tannins in the plant samples

Compounds	*C. officinalis* (Bark)			Standard
	1	2	3	
Alkaloids	0.96	0.73	-	0.94
Phenols	0.98	0.8	-	0.96
Flavonoids	0.94	0.34	-	0.94
Tannins	0.92	0.65	0.26	0.91

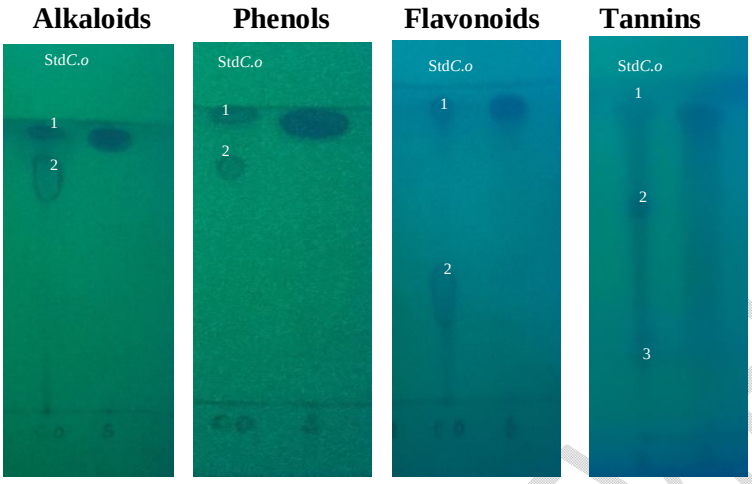

Figure 1: TLC separation of alkaloids, phenols, flavonoids and tannins of *C. officinalis* bark methanol extract

The spots (Compounds) observed on TLC plates for *C. officinalis* are 1 and 2 in case of alkaloids, phenols and flavonoids and 3 in tannins. RF value range 0 indicates the solvent polarity is very low and 1 indicating solvent polarity is very high. The stronger a compound is bound to the adsorbent, the slower it moves up the TLC plate. A non-polar compound moves up the plate more rapidly (higher RF value) than the polar compounds (lower RF value). The RF value so obtained for all the compounds in average is very close to 1 which means the polarity of the compounds is very low (non- polar compounds).

3.2.High Performance Liquid Chromatography Analysis

Pratiwi*et al*., (2018) reported that the commercially vital alkaloid quinine was isolated from cell cultures of Cinchona species. HPLC has proved as one such most useful technique used for the separation of quinoline alkaloids. The HPLC chromatogram denoted the prominent peaks of the eluted compounds of *C. officinalis* (Bark) in the retention time of 1.750, 2.250, 4.016 and 7.266 minutes. The eluted compounds were identified as quinine, isoquinidine and cinchonidine. Reece and Peikert (1980) used HPLC method for rapid quantification of plasma levels of quinidine, dihydroquinidine and 3-hydroxyquinidine. Similarly, Murauer and Ganzera (2018) isolated dihydroquinidine, dihydroquinine, quinidine, quinine, cinchonine and cinchonidine from the bark of cinchona.

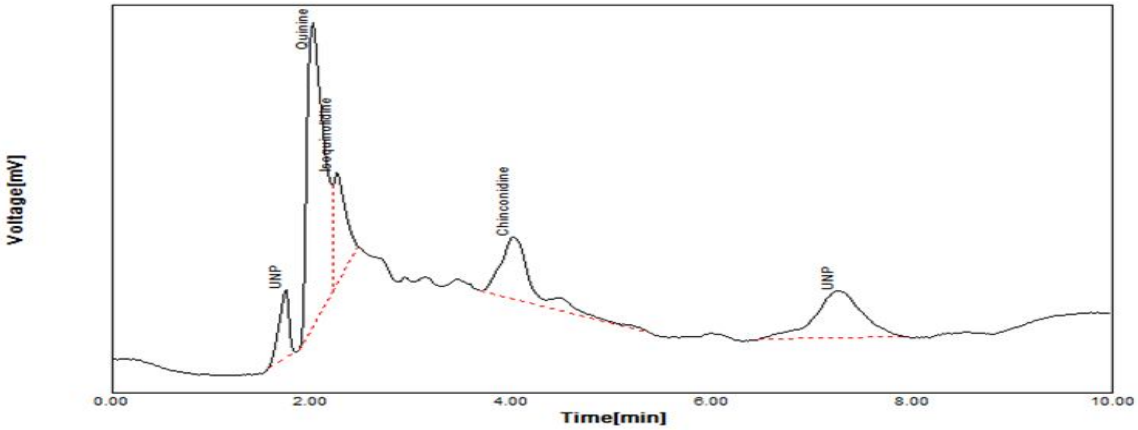

Figure :2. HPLCChromatogram plot of *C. officinalis* (Bark)

3.3.Gas Chromatography and Mass Spectrometry Analysis

The phytocompounds in the plant samples were characterised based on the interpretation of mass spectrum of GC-MS/MS in reference with the database of National Institute of Standard and Technology (NIST). The NIST 20 EI Library has 350,643 spectra for 306,869 compounds and over 447 k literature and experimentally determined GC methods and Retention indexes for 139,963 compounds. The name, molecular weight and structure of the components in the plant samples were ascertained by comparing the spectrum of unknown compounds with the spectrum of known compounds stored in the NIST library. Gas chromatography and Mass spectroscopy analysis of methanol extract of *C. officinalis* (Bark) was performed.

The chromatogram denoted the prominent peaks of the eluted compounds of *C. officinalis* (Bark) in the retention time of 9.569, 10.488, 11.814, 13.593, 14.282, 16.542, 16.967, 23.494, 24.954, 25.151, 25.988, 26.752, 26.982, 27.906, 28.277, 31.454, 33.604, and 35.744 minutes. By correlating the retention times with (NIST) mass spectral library the eluted compounds were confirmed as Phthalic acid, Hexadecanoic acid,1,2,3,5-Cyclohexanetetrol, Cinchonab-9-one, 9,12-Octadecadienoic acid (Z,Z)-, Heptadecanoic acid, Quinine, Cinchonine, Cinchonidine, Phenol, Quinine, and gamma-Sitosterol.The eluted bioactive compounds present in the plant samples are responsible for their antimicrobial, antioxidant, anti-inflammatory and anti- arthritic activity. Phytotherapies approved for clinical treatments motivates the search for plant-based phytochemicals with therapeutic potential (PiovezanaBossolani et al., 2018). The botanicals especially flavonoids such as luteolin and by β-sitosterol isolated from the methanol extract of *Cissusquadrangularis* stem reported to have anti-inflammatory activity Rasale, (2014).

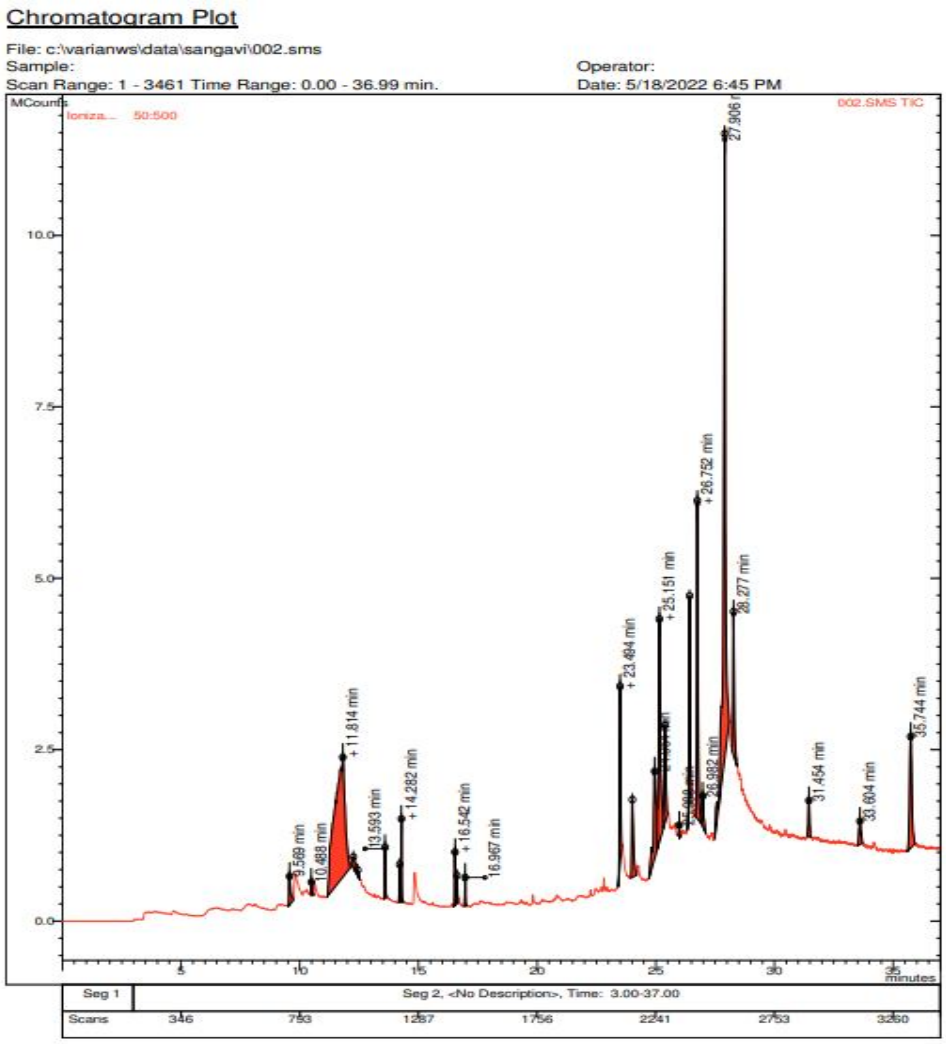

Figure :3. GC-MS/ Chromatogram plot of bark of *C. officinalis*

3.4.Investigation of anti- Inflammatory Activity of bark extract of *C. officinalis*.

Anti- inflammatory activity of bark extract of *C. officinalis* (75 mg/ ml concentration) was evaluated by protein (BSA) denaturation method using diclofenac (75 mg/ ml concentration) as positive control. The methanol extract of*C.officinalis* (Bark) have showed anti- inflammatory properties of range between 17.0 and 57.3%.Among the phytochemical components isolated in plants, flavonoids (Serafini*et al*., 2010), alkaloids (Liu *et al*., 2018) and saponins (Lee *et al*., 2018) are reported to have effective anti-inflammatory activity. This activity is attributed to the greater antioxidant properties of polyphenols and presence of alkaloids in the plant samples. Hence these natural active compounds can be used as an alternative to synthetic, steroidal and non- steroidal drugs available in the market to treat pain and inflammation.

Table:3. Anti-inflammatory activity of Diclofenac and *C.officinalis* (Bark)

Concentration	Diclofenac (%)	*C. officinalis* (Bark)- %
20 µl	42.6	17.0
40 µl	53.2	20.6
60 µl	62.0	28.7
80 µl	70.5	49.2
100 µl	89.8	57.3

3.5.Investigation ofAnti- Arthritic Activity of bark extract of *C. officinalis*.

Anti- arthritic activity of bark extract of *C. officinalis* (75 mg/ ml concentration) was evaluated by albumin denaturation method using diclofenac (75 mg/ ml concentration) as positive control. The methanol extract of *C.officinalis* (Bark) have showed anti- arthritic properties of range between 18.4- 58.5%. Based on the results obtained it was confirmed that the bioactive compounds present in the bark extract of *C. officinalis*inhibit the denaturation of proteins. Thus, the bark extract of *C. officinalis*can be used to treat arthritissubject to animal and human studies. Wang et al., 2012 observed that the arthritis activity of *Urticaatrichocaulis*was the combined action of multiple phenolic constituents.Souto *et al*., (2011) observed that ~~the~~ alkaloids such as isoquinoline, colchicines and aconitine isolated from plants werefound effective ~~against~~forarthritis treatment. Phytochemical compounds present in *C. officinalis*are most related for treatment of Arthritis.

Table:4. Anti-arthritic activity of Diclofenac, and *C.officinalis* (Bark)

Concentration	Diclofenac (%)	*C. officinalis* (Bark)- %
20 µl	49.2	18.4
40 µl	54.6	23.7
60 µl	63.7	30.4
80 µl	71.3	49.8
100 µl	90.8	58.5

Conclusion

The bioactive compounds ~~Quinone in particular~~present in the methanolic extract of bark of *C. officinalis*are responsible for various biological properties including anti-arthritic activity. The study revealed the anti-inflammatory and anti-arthritic properties of bark extract of *C.officinalis* by inhibiting the denaturation of proteins. ~~Based on the results it was confirmed that the activities of *C.officinalis* (Bark) is more when compared to *C. quadrangularis* (Stem) and *W. tinctoria* (Leaf).~~ All the active ingredients present in bark of *cinchona* was cited for the anti-arthritic activity. Therefore, the bark extract of *C.officinalis*was used in the development of therapeutic product. The present work concludes to recommend to develop the formulation of an aerosol for arthritis using natural ingredients and the product is under development at Ceras Pharmaceuticals Private Limited. The product will be further tested among healthy individuals for its efficiency and toxicity.

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2.

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