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Biocure: Herbal healthcare for inflammatory and arthritic diseases

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Abstract

Plant based cure for the infectious diseases is out bursting due to their therapeutic potential in human population's great interest towards herbal healthcare. This study aimed at investigation on the preventive potential of Wrightia tinctoria, Cissus quadrangularis and Cinchona officinalis for inflammation and arthritis with the identification of potential biologically active compounds by GC/MS/MS chromatographic analysis and evaluation of their antioxidant, anti-inflammatory and anti-arthritis activities. The phytocompounds Quinine, Cinchonine, Phytol, Octadecanoic acid methyl ester, Stigmasterol etc. were identified as potential compounds from the above mentioned plants. Further, the evaluation of the methanol extracts of the plant samples exhibited significant antioxidant /free radical scavenging activity (62.18- 69.37 %), (60.98- 69.50 %), (70.57- 74.43 %), anti-inflammatory activity ranges (2.6- 54.1%), (10.2- 56.2%), (17.0- 57.3%) and anti- arthritic activity ranges (4- 55.8%), (11.6- 57.1%), 18.4- 58.5%) in par with the standards, confirmed that the bioactive compounds present in the said plant extracts inhibit the denaturation of proteins, a well-documented cause of inflammation and rheumatoid arthritis. The result indicated that plant samples cab be considered as an alternate herbal sources for anti-inflammatory and anti-arthritic healthcare products.

Keywords: Wrightia tinctoria, Cissus quadrangularis, Cinchona officinalis, chromatography, phytocompounds, antioxidant, anti-inflammatory, anti-arthritis

Introduction

Inflammation is a biological response of vascular tissues and a defense mechanism that intended to remove the injurious stimuli such as pathogens, damaged cells or irritants either physical or chemical (Maldini et al., 2009). Prolonged inflammation may lead to reactive arthritis, a bilateral disease especially rheumatoid arthritis (RA), a widespread chronic inflammatory systemic autoimmune disease of unknown etiology which leads to hyperplasia, vasculogenesis, cartilage and bone destruction, joint malformation, functional impairment (Chunxia et al., 2011).WHO reported that 0.3-1% of the world population is affected from rheumatoid arthritis and among them females are three times more prone to the disease as compared to males (Tripathy et al., 2010). Arthritis is the most common cause of disability in adults all over the world, restricting routine activities for billions of people. It is triggered by genetic

predisposition, exposure to virus and release of certain free radicals (nitrous oxide and superoxide radicals) which may induce the T-cells to produce the inflammatory cytokines, such as interleukins (IL) and tumor necrosis factor (TNF-α) thereby influenced the production of growth factors, cytokines and adhesive molecules on immune cells the causative for inflammation (Kasper et al., 2005) and the modulation of their production may be an effective therapy.

Nonsteroidal anti-inflammatory drugs (NSAIDs), the cyclooxygenase (COX) inhibitors are often usedto fight against pain and inflammatory disorders (Abdel-Aziz et al., 2011). NSAIDs use is limited due to their adverse effects such as myocardial infarction, increase in high blood pressure, gastric irritation, liver and kidney problems, loose stool, nausea, vomiting, heart failure and dyspepsia if no antibiotic is associated (Guo et al., 2011). Generic drugs are more expensive which many developing countries can't afford. In recent years, to overcome these obstacles, great interest have aroused towards the use of systematic traditional herbal medicines due to their therapeutic potential either directly as folk medication or indirectly in the preparation of recent pharmaceuticals. In India, more than 2500 plants species are the primary source of healthcare and used as herbal medicaments for nearly 85% of the global population (Pešić, 2015). 40% of synthetic drugs available in pharmaceutical markets are derived from plants and microbial-based natural products (Bauer & Brönstrup, 2014). Hence, anti-inflammatory substances from various plant sources could be an alternative source. Wrightia tinctoria,Cissus quadrangularis and Cinchona officinalis are such plant reported to have significant anti-inflammatory activity (Tharkar et al., 2009; Battu Ganga Rao et al., 2019: Siddiqua et al., 2017). Anti-inflammatory activity reported in Cissus quadrangularis is due to luteolin and by β-sitosterol (Siddiqua et al., 2017) and for centuries Cinchona officinalis have been used for medicinal purposes (Raza et al., 2021).Hence, present work focused to identify and isolate bioactive compound with potential anti-inflammatory and anti-arthritis activities as Biocure from plant sources Wrightia tinctoria, Cissus quadrangularis and Cinchona officinalis.

Methodology

Plant material

The leafsample of W.tinctoria was collected from Kailasampalayam, Thiruchengode, Namakkal district, North western zone of Tamilnadu situated between 11°103'46.69" N latitude and 76°81'91.44" E longitude; the stem of C. quadrangularis was collected from Mundandurai Tiger Reserve, Tirunelveli district, Southern zone of Tamilnadu located between $8^0$66'00.60"N latitude and $77^0$32'75.00"E longitude and the bark of C. officinalis was collected from the original sources from Cinchona Village, Doddabetta, The Nilgiris district, Hilly Zone of Tamil Nadu situated between 11º25'37" N and 76º43'48"E. The plant samples were brought to the laboratory, washed under running tap water, shade dried at room temperature, ground into fine powder and stored for further experimental analysis.

Preparation of the extracts

The powdered plant samples of W. tinctoria leaf, stem of C. quadrangularis and bark of C. officinalis (20 g each) were subjected to hot extraction with 350 ml of methanol using Soxhlet apparatus at 65-80°C for 8-10 h. The solvents were evaporated in rotary vacuum evaporator until complete removal of solvents and stored at 4°C for further use. The stored plant extracts were then dissolved in respective solvents while experimentation to get the solution of 10 mg/10 ml.

Qualitative screening of phytochemicals

Qualitative phytochemical screening was carried out to identify the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, saponins, sterols, phenols, glycosides, and terpenoids present in the leaves, stem, and bark samples of W. tinctoria,C. quadrangularis and C. officinalis respectively using standard methods (Harborne, 1967).

Quantitative analysis of phytochemicals

Quantitative evaluation was done to quantify or identify the concentration of phytochemicals present in the leaf, stem and bark of W. tinctoria, C. quadrangularis and C. officinalis respectively. The estimation of proteins **(**Satpathy et al., 2020), alkaloids (Shamsa et al., 2008), phenols (Babaet al., 2015), flavonoids (Madhu et al., 2016), steroids (Nair et al., 2021), saponins (Madhu et al., 2016) and tannins (CI et al., 2016) were performed using the standard protocols.

GC/MS/MS analysis

The GC-MS/MS analysis was performed using Varian 3800 with Mass spectrum 4000 GC-MS/MS system equipped with a Fused silica capillary column of size 15m x 0.2 mm ID x 1µm linked to an EI detector. Helium gas (99.99% purity) was used as a carrier gas at a constant flow rate of 1ml/min and the sample injected was 1µl; the instrument was set to an initial temperature of 110 ºC, and maintained at this temperature for 2 min. At the end of this period the oven temperature rose up to 280 ºC, at the rate of an increase of 5 ºC/min, and maintained for 9 min. Injection port temperature was ensured as 250 ºC. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS/MS compounds present in the plants sample were identified.The name, molecular weight and structure of the components of the test materials were ascertained.

Evaluation of Antioxidant Activity

The antioxidant activity of the plant extracts were estimated using DPPH Assay measuring the scavenging capacity of antioxidants (Shekhar et al., 2014). Different volumes (20 µl, 40 µl, 60 µl, 80 µl and 100 µl) of plant extracts (100mg/ ml concentration) and standard solution were made upto 100 µl with DMSO and 2.9 ml DPPH (0.1 mM) solution was added to each test tube. The reaction mixture was shaken well and incubated in dark condition at room temperature for about 20 minutes. 3 ml of DPPH solution was taken as control. The absorbance of the mixture was read at 517 nm and calculated the antioxidant activity.

Evaluation of Anti- Inflammatory Activity

The anti- inflammatory activity of plant extracts were evaluated using protein denaturation method(Williams et al., 2008). Different volumes (20 µl, 40 µl, 60 µl, 80 µl and 100 µl) of plant extracts (75 mg/ ml concentration) and standard solution (Diclofenac 75mg/ml)were made upto 100 µl with methanol. 5ml of 0.2% w/v BSA solution were added. The test tubes were heated at 72ºC for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined by using UV- Visible spectrophotometer at a wave length of 660 nm against the blank. The % inhibition of precipitation (denaturation of the protein) was determined on a % basis relative to the control. .

% inhibition = [(Abs control - Abs sample)/ Abs control] x 100

Evaluation of Anti- Arthritic Activity

The anti- arthritic activity of plant extracts were evaluated using albumin denaturation method (Vaijanthimala et al., 2019). Different volumes (20 µl, 40 µl, 60 µl, 80 µl and 100 µl) of plant extracts (75 mg/ ml

concentration) and standard solution were made upto 100 µl with methanol. 0.2 ml of egg albumin and 2.8 ml of phosphate buffer saline was added to each test tube. The P^H of the above solutions was adjusted to 6.4 using a small amount of 1 N HCl. The solutions were incubated at 37ºC for 20 minutes and heated at 70ºC for 5 minutes. After cooling, the absorbance of the solutions was determined by using UV- Visible spectrophotometer at a wave length of 660 nm against the blank. The % inhibition of precipitation (denaturation of the protein) was determined on a % basis relative to the control.

% inhibition = [(Abs control - Abs sample)/ Abs control] x 100

Results

The leaf of W. tinctoria, stem of C. quadrangularis and bark of C. officinalis extracted with methanol yielded 63.64%, 69.6% and 71.1% respectively.Qualitative evaluation of phytochemicals in the methanol extract of leaf, stem and bark samples of W. tinctoria, C.quadrangularis and C.officinalis revealed the presence of plant metabolites viz., proteins, alkaloids, phenols, flavonoids, glycosides, cardiac glycosides, steroids, Terpenoids, Sterols, Quinones, Saponins and tannins (Table 1).

Table 1: Qualitative screening of phytochemicals in methanol extract of W. tinctoria (Leaves), C. quadrangularis (Stem) and C. officinalis (Bark).

Sn.	Phytochemical constituents	Conformation test	Name of plant species		
			W. tinctoria	C. quadrangularis	C. officinalis
1	Proteins	Xanthoproteic Test	+	+	+
2	Carbohydrates	Fehling's Test	-	-	-
3	Alkaloids	Mayer's Test	+	+	+
4	Phenols	Lead acetate Test	+	+	+
5	Flavonoids	H_2SO_4 Test	+	+	+
6	Anthocyanins	HCL Test	-	-	-
7	Glycosides	NaOH Test	+	+	+
8	Cardiac Glycosides	Salkowski Test	+	+	+
9	Steroids	Salkowski Test	+	+	+
10	Terpenoids	Salkowski Test	+	+	+
11	Sterols	Salkowski Test	+	+	+
12	Quinones	H_2SO_4 Test	+	+	+

Note:'+'indicatesPositive , '-' indicates Negative

Quantitative evaluation of the methanol extract of leaf sample of W. tinctoria, stem sample of C. quadrangularis and bark sample of C. officinalisrevealedthe phytochemicals were comparatively more in C. officinalis followed by C. quadrangularis and W. tinctoria the least (Table 2). Protein content was very high (60 %) in bark ofC. officinalis (33.4 mg/g) followed by stem of C. quadrangularis (13.70 mg/g) and leaves of W. tinctoria (11.25 mg/g).Steroid, flavonoids and tannins were almost 50-60 % high in bark ofC. officinalisexcept saponins,

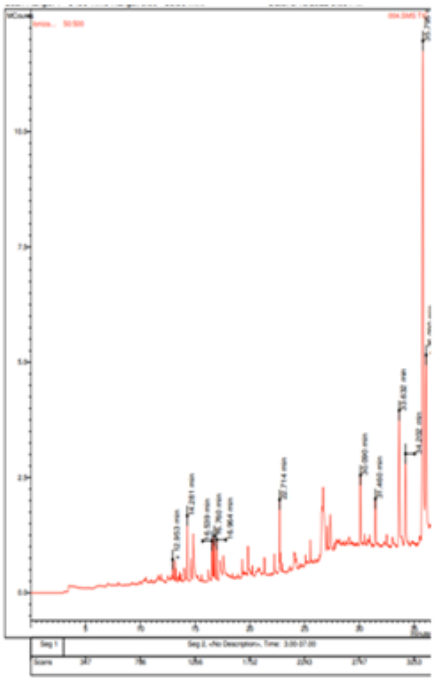
reported high in C. quadrangularis and not reported in W. tinctoria.

Table 2: Quantitative analysis of phytochemicals in methanol extract of W. tinctoria (Leaves), C. quadrangularis (Stem) and C. officinalis (Bark)

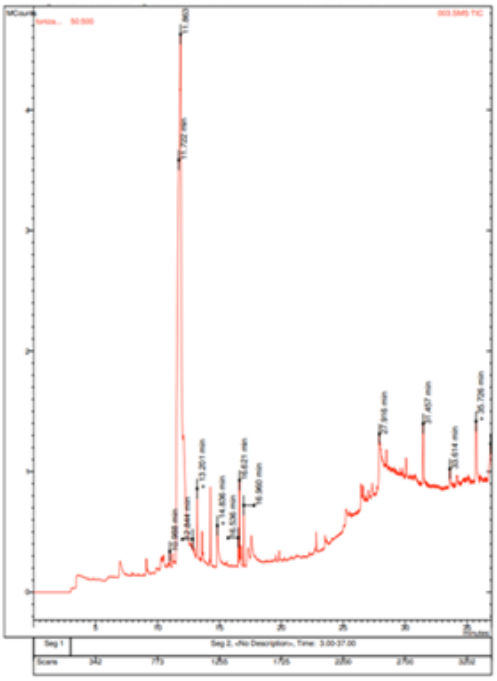
Plant Metabolites	W. tinctoria (Leaves)	C. quadrangularis (Stem)	C. officinalis (Bark)
Proteins (mg\g)	11.25	13.70	33.400
Alkaloids (mg\g)	0.121	0.148	0.235
Phenols (mg\g)	0.356	0.316	0.817
Flavonoids (µg\g)	0.15	0.48	1.6
Steroids (mg\g)	1.944	0.518	2.858
Saponins (mg\g)	-	0.131	0.127
Tannins (mg\g)	12.774	12.726	22.790

GC/MS/MS analysis of plant samplesGC-MS analysis of the methanol extracts of W. tinctorialeaf, C. quadrangularis stem and C. officinaliselicited various phytocompounds with biological activities such as Hypocholesterolemic, antimicrobial, anticancer, diuretic, antifungal, joint dislocation, stimulant, antimalarial, antioxidant, anti-arthritis, anti-inflammatory properties etc. The compound gamma.-Sitosterol elicited at Rt 35.795 in highest concentration from W. tinctoria followed by 12-Oleanen-3-yl acetate, (3.alpha.)-, Stigmasterol and Campesterol (Table 3). 2,3-Di-O-methyl-D-xylopyranose was resolved high from C. quadrangularis (Table 4) andQuinine the maximum from C. officinalis bark (Table 5). Five compounds identified in the leaf of W. tinctoria 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; Phytol; Octadecanoic acid methyl ester; Stigmasterol and 12-Oleanen-3-yl acetate, (3.alpha.); 5 compounds in the stem of C. quadrangularis 2,3-Di-O-methyl-D-xylopyranose; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester and 6 compounds in the bark of C. officinalis Quinine, Cinchonine, Cinchona-9-one, 6'-methoxy-, (8.alpha.) and Phenol, 2,6-bis(bicyclo[2.2.1]hept-2-yl)-4-(1,1-dimethyl ethyl) found to have anti-inflammatory activity. Quinine and stigmasterol reported to have anti-arthritis activity. Other than these many compounds Aspidospermidine-3-carboxylic acid, 2,3-didehydro-1-methyl-, methyl ester, (5.alpha., 12.beta., 19.alpha.)-; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; phytol, Docosanoic acid, methyl ester, Campesterol, stigmasterol, gamma.-Sitosterol, 12-Oleanen-3-yl acetate, (3.alpha.) identified in the leaf extract of W. tinctoria, Pentadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester. Quinine, Gamma.-Sitosterol identified in the stem extract of C. quadrangularisand 1,2,3,5-Cyclohexanetetrol (1.alpha.,2.beta.,3.alpha.,5.beta.)-, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, Quinine, Gamma.-Sitosterol identified from the bark extract of C. officinalis reported to have anti-cancer activity.

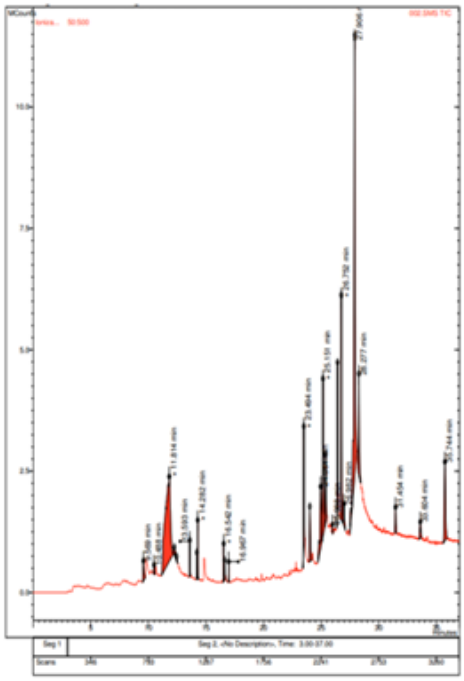
Graph 1: GC/MS/MS chromatogram of W. tinctoria leaf, C. quadrangularis stem and C. officinalis bark



GC/MS/ MS chromatogram *W. tinctoria* leaf



GC/MS/ MS Chromatogram of *C. quadrangularis* stem



GC/MS/ MS Chromatogram of *C. officinalis* bark

Table 3: Compounds identified from leaves of W. tinctoria by GC-MS/MS analysis

Sn.	RT (Min)	Area (%)	Name of Compound	Molecular weight	Molecular Formula	Biological Activity
1	12.953	1.744e+6	Aspidospermidine-3-carboxylic acid, 2,3-didehydro-1-methyl-, methyl ester, (5.alpha.,12. beta., 19. alpha.)-	352	$C_{22}H_{28}N_2O_2$	Anticancer activity.
2	14.281	2.779e+6	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	Antioxidant, antimicrobial, nematicide, pesticide, flavor and antiandrogenic activity.
3	16.539	1.692e+6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294	$C_{19}H_{34}O_2$	Antiinflammatory,hypocholesterolemic, anticancer, and hepatoprotective activity.
4	16.760	2.074e+6	Phytol	296	$C_{20}H_{40}O$	Hypocholesterolemic, antimicrobial, anticancer, diuretic, antifungal, joint dislocation, stimulant, antimalarial and anti-inflammatory properties.
5	16.964	1.921e+6	Octadecanoic acid, methyl ester	298	$C_{19}H_{38}O_2$	Anti-inflammatory, Cosmetics and used to produce dietary supplements.
6	22.714	3.429e+6	Docosanoic acid, methyl ester	354	$C_{23}H_{46}O_2$	Antimicrobial and antioxidant activity
7	33.632	1.692e+7	Campesterol	400	$C_{28}H_{48}O$	Anti-cancerous and anti-tumor properties
8	34.202	1.208e+7	Stigmasterol	412	$C_{29}H_{48}O$	Antioxidant, hypoglycaemic and thyroid inhibiting properties, precursor of progesterone, antimicrobial, anticancer, anti-arthritic, anti-asthma, anti-inflammatory and diuretic properties.
9	35.795	7.427e+7	.gamma.-Sitosterol	414	$C_{29}H_{50}O$	Anti-hyperglycemic and anticancer activity.
10	36.090	2.804e+7	12-Oleanen-3-yl acetate, (3.alpha.)-	468	$C_{32}H_{52}O_2$	Antibacterial, anthelmintic, anti-inflammatory, hepatoprotective, immune potential, antipyretic, antioxidant, antifungal, anticancer, and anti-HIV activity.

Table 4: Compounds identified from stem of C. quadrangularis by GC-MS/ MS analysis

Sn.	RT (min)	Area (%)	Name of the compound	Molecular weight	Molecular Formula	Biological activity
1	10.988	394712	Megastigmatrienone	190	$C_{13}H_{18}O$	Antitumor, aroma, Antioxidant and Antimicrobial activity.
2	11.722	2.557e+7	2,3-Di-O-methyl-D-xylopyranose	178	$C_7H_{14}O_5$	Antioxidant, anti-inflammatory and antimicrobial activity.
3	11.863	4.114e+7	2,3-Di-O-methyl-D-xylopyranose	178	$C_7H_{14}O_5$	Antioxidant, anti-inflammatory and antimicrobial activity.
4	13.201	1.557e+6	Decahydro-naphthalene-1,8a-diol	170	$C_{10}H_{18}O_2$	Antioxidant, aroma and antimicrobial activity.
5	14.277	1.299e+6	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	Antioxidant, antimicrobial, nematicide, pesticide, flavor and antiandrogenic activity.
6	14.836	1.366e+6	Pentadecanoic acid	242	$C_{15}H_{30}O_2$	Antimicrobial, antioxidant, anti-asthmatics and anticancer properties.
7	16.536	489912	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294	$C_{19}H_{34}O_2$	Anti-inflammatory, hypocholesterolemic, anticancer, and hepatoprotective properties.
8	16.621	1.667e+6	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	356	$C_{21}H_{40}O_4$	Anti-inflammatory, antiandrogenic, cancer preventive, hypocholesterolemic, 5-Alpha reductase inhibitor, antimutagenic, insectifuge and flavor.
9	16.960	1.227e+6	Heptadecanoic acid, 15-methyl-, methyl ester	298	$C_{19}H_{38}O_2$	Antimicrobial and antioxidant activity.
10	27.916	810991	Quinine	324	$C_{20}H_{24}N_2O_2$	Antiprotozoal, anticancer, antimicrobial, anti-inflammatory, anti-arthritic, antiparasitic and antiviral activity.
11	31.457	2.110e+6	Manganese, dicarbonyl [(1, 2, 3, 3a,7a-.eta.)-1H-inden-1yl] (triphenylphosphine)	488	$C_{29}H_{22}MnO_2P$	Antioxidant, Antiparasitic and anti-bacterial activity.
12	35.726	3.044e+6	Gamma.-Sitosterol	414	$C_{29}H_{50}O$	Anti-hyperglycemic and anticancer activity.

Table 5: Compounds identified from bark of C. officinalis by GC-MS/MS analysis

Sn.	RT (min)	Area (%)	Name of the compound	Molecular weight	Molecular Formula	Biological activity
1	11.814	5.483e+7	1,2,3,5-Cyclohexanetetrol (1.alpha.,2.beta.,3.alpha.,5.beta.)-	148	$C_6H_{12}O_4$	Anesthetic, antioxidant, antiseptic, antibacterial, antiviral and cancer preventive.
2	14.282	2.654e+6	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	Antioxidant, antimicrobial, nematicide, pesticide, flavour and antiandrogenic activity.
3	16.542	1.767e+6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294	$C_{19}H_{34}O_2$	Anti-inflammatory, hypocholesterolemic, anticancer, and hepatoprotective activity.
4	16.967	943933	Heptadecanoic acid, 16-methyl-, methyl ester	298	$C_{19}H_{38}O_2$	Antimicrobial and antioxidant activity.
5	23.494	8.662e+6	Quinine	324	$C_{20}H_{24}N_2O_2$	Antiprotozoal, anticancer, antimicrobial, anti-inflammatory, anti-arthritic, anti-parasitic and antiviral properties.
6	24.954	1.107e+7	Cinchonine	294	$C_{19}H_{22}N_2O$	Antimalarial, stimulant of gastric mucosa, used in rheumatism and neuralgia, anti-obesity, anti-parasitic and anti-inflammatory activity.
7	25.151	1.791e+7	Cinchonidine	294	$C_{19}H_{22}N_2O$	Anti-parasitic and anti-malarial activity.
8	26.423	1.015e+7	Cinchona-9-one, 6'-methoxy-, (8.alpha.)-	322	$C_{20}H_{22}N_2O_2$	Antimicrobial, anti-malarial and anti-inflammatory activities.
9	26.752	1.639e+7	Phenol, 2,6-bis(bicyclo[2.2.1]hept-2-yl)-4-(1,1-dimethyl ethyl)-	338	$C_{24}H_{34}O$	Antioxidant, anti-inflammatory, cytotoxicity, antimicrobial, Phytotoxicity, insecticidal and nematicidal activities.
10	27.906	5.448e+7	Quinine	324	$C_{20}H_{24}N_2O_2$	Anti-protozoal, anticancer, antimicrobial, antioxidant, anti-inflammatory, anti-arthritic, anti-parasitic and antiviral activity.
11	35.744	1.075e+7	.gamma.-Sitosterol	414	$C_{29}H_{50}O$	Anti-hyperglycemic and anticancer activity.

Antioxidant Activity of plant extracts.

Epidemiological studies have been reported that many of the antioxidant compounds possesses anti- inflammatory, antimutagenic, anticarcinogenic, antiviral and antibacterial activities to greater or lesser extent. It has been reported that antioxidants modulate the pathophysiology of chronic inflammation up to some extent. Antioxidant activity in methanol extract of plant samples (100mg/ ml concentration) determined using DPPH assay with ascorbic acid (100mg/ ml concentration) as positive control showed that W. tinctoria (Leaf), C. quadrangularis (Stem) and C.officinalis (Bark) exhibited significant antioxidant /free radical scavenging activity of 62.18- 69.37 %, 60.98- 69.50 % and 70.57- 74.43 % respectively. Free radical scavenging activity was high in C. officinalis (74.43 %). Overall all the plant samples showed significant antioxidant activity.

Table 6: Antioxidant activity of methanol extract of W. tinctorialeaves, C. quadrangularisstem and C.officinalis bark

Concentration	Ascorbic acid (%)	W. tinctoria (Leaves)- %	C. quadrangularis (Stem)- %	C. officinalis (Bark)- %
20 µl	85.35	62.18	60.98	70.57
40 µl	87.48	62.58	66.04	71.37
60 µl	88.01	64.18	67.64	72.48
80 µl	88.94	65.77	68.57	73.10
100 µl	89.88	69.37	69.50	74.43

Anti- Inflammatory Activity of **plant extracts.**

Anti- inflammatory activity of the plant extracts evaluated showed that the methanol extract of W. tinctoria leaves, C. quadrangularis stem and C. officinalis bark found to have anti- inflammatory properties ranging from 2.6- 54.1%, 10.2- 56.2% and 17.0- 57.3% respectively. W. tinctoria leaves, C. quadrangularis stem and C. officinalis bark samples observed to have significant anti-inflammatory activity at 100 µl concentration. The activity of plant extracts is attributed to the greater antioxidant properties of polyphenols and presence of alkaloids in the plant samples.

Table 7: Anti-inflammatory activity of methanol extract of W. tinctorialeaves, C. quadrangularisstem) and C.officinalis bark samples

Concentration	Diclofenac (%)	W. tinctoria (Leaves)- %	C. quadrangularis (Stem)- %	C. officinalis (Bark)- %
20 µl	42.6	2.6	10.2	17.0
40 µl	53.2	15.5	19.5	20.6
60 µl	62.0	27.5	28.0	28.7
80 µl	70.5	46.2	47.7	49.2
100 µl	89.8	54.1	56.2	57.3

Anti- Arthritic Activity of plant extracts.

The methanol extract of W. tinctorialeaf, C. quadrangularisstem and C.officinalis bark have showed anti- arthritic properties of ranges 4- 55.8%, 11.6- 57.1% and 18.4- 58.5%. Based on the results obtained it was confirmed that the bioactive compounds present in the

plant extracts inhibit the denaturation of proteins. Thus the W. tinctoria (Leaf), C. quadrangularis (Stem) and C. officinalis (Bark) can be used to treat arthritis.

Table 8: Anti-arthritic activitiesof W. tinctorialeaves, C. quadrangularisstem and C.officinalis bark

Concentration	Diclofenac (%)	W. tinctoria (Leaves)- %	C. quadrangularis (Stem)- %	C. officinalis (Bark)- %
20 µl	49.2	4.0	11.6	18.4
40 µl	54.6	28.7	20.1	23.7
60 µl	63.7	28.7	29.5	30.4
80 µl	71.3	47.5	48.3	49.8
100 µl	90.8	55.8	57.1	58.5

Discussion

Plant samples such as Wrightia tinctoria (leaf), Cissus quadrangularis (stem) and Cinchona officinalis(bark) werereported to have significant anti-inflammatory activities. Plant samples werescreened for the phytocompound with potential biological activities, which could be a herbal source for anti-inflammatory and anti-arthritis activity. Observed the presence of plant metabolites such as proteins, alkaloids, phenols, flavonoids, glycosides, cardiac glycosides, steroids, terpenoids, sterols, quinones, saponins and tannins. All phytochemicals were quantified comparatively high in C. officinalis followed by C. quadrangularis and W. tinctoria the least. Proteins, steroid, flavonoids, phenols, alkaloid and tannins were almost 50-60 % high in bark ofC. officinalis except saponins, reported high in C. quadrangularis and not reported in W. tinctoria. Phenolics derived from various natural sources are associated to antioxidant, anti- inflammatory, anti-allergic, anti- arthritic and antimicrobial activities. The organic molecules from plants secondary metabolism such as terpenoids, polyphenols, alkaloids, flavonoids, lignins and tannins reported to have potential antioxidant and antibacterial activity (Agbor et al., 2011). Due to the anti-oxidative, antimicrobial and free radical scavenging properties phenolics promote excellent healing of wounds (Deshmukh et al., 2009). Plants as rich source of flavonoids, alkaloids and terpenoids found to have anti-inflammatory, analgesic and antioxidant activity (Paschke et al., 2013).

GC-MS analysis of the methanol extracts of W. tinctoria leaf, C. quadrangularis stem and C. officinalisbarkelicited various phytocompounds with biological activities such as Hypocholesterolemic, antimicrobial, anticancer, diuretic, antifungal, joint dislocation, stimulant, antioxidant, antimalarial, anti-inflammatory properties etc. Stigmasterol was elicited maximum from W. tinctoria and Quinineelicited maximum from C. officinalis. Stigmasterol, a main plant sterol reported to have potential pharmacological activity including anti-arthritis and anti-inflammatory activities(Gabay et al. 2010; Chen et al. 2012).The methanol extract of the W. tinctoria leaves, C. quadrangularis stem and C.officinalis bark exhibited significant antioxidant /free radical scavenging activity of 62.18- 69.37 %, 60.98-69.50 % and 70.57- 74.43 %, anti- inflammatory properties of ranges2.6- 54.1%, 10.2- 56.2% and 17.0-57.3% and anti- arthritic properties of range 4- 55.8%, 11.6- 57.1% and 18.4- 58.5%.Based on the results obtained it was confirmed that the bioactive compounds present in the plant extracts inhibited the denaturation of proteins which is a well-documented cause of

inflammation and rheumatoid arthritis (Patil et al., 2019). Thus the W. tinctoria (Leaves), C. quadrangularis (Stem) and C. officinalis (Bark) can be used to treat inflammation and arthritis. Denaturation of proteins is a renowned root of inflammation and arthritis. Vaijayanthimala et al., (2019) showed that the methanol extract of stem of C. quadrangularis showed potent anti-arthritic property by inhibiting 16.47- 82.35% denaturation of egg albumin proteins. Phenols, flavonoids, alkaloids, steroids and terpenoids present in the plant samples of the studied species may be responsible for their potential antioxidant, anti-inflammatory and anti-arthritis activities. Strong anti-inflammatory activity of flavonoids is desirable for the management of chronic inflammatory diseases (Aquila et al., 2009). Rasale, (2014) observed anti-inflammatory activity in methanol extract of stem of C. quadrangularis due to the presence of flavonoids especially luteolin and by β-sitosterol.Tri terpenoids inhibit the histamine release which is vital in producing inflammation and thereby exerts antiinflammatory activity (Adeneye et al., 2014). Saponins and alkaloids exerts antiinflammatory activity via down regulating TNF-α in the inflammatory tissues (Karthik et al., 2016; Alamgeer et al., 2017). The alkaloids such as isoquinoline, colchicines and aconitine isolated from plants are found effective for arthritis treatment (Barbosa-Filho et al., 2006; Souto et al., 2011). In the present study it is substantiated that the compounds reported in the plants samples accounts for their significant biological activities and hence, these plant sources may be considered as a potential herbal health care for anti-inflammatory and anti-arthritis properties. .

Conclusion

The bioactive compounds present in W. tinctorialeaf, C. quadrangularisstem and C. officinalisbark may be responsible for various biological properties. The methanol extract of all three plant samples showed potent antioxidant/ radical scavenging activity due to the presence of high amount of polyphenols and alkaloids. The bioactive compounds present in the plant extracts inhibit the denaturation of proteins which is a well-documented cause of inflammation and rheumatoid arthritis. Some of the active ingredients present in bark of cinchona were cited for the anti-arthritic activity. Hence, W. tinctoria leaf, C. quadrangularis stem and C. officinalisbark having bioactive compounds with potential antioxidant, anti-inflammatory and anti-arthritis activities can be used in the development of an efficient herbal health care products.

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