

Role of *Achyranthes aspera* in Rheumatoid Arthritis Treatment

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Abstract: GC-MS analysis of the ethanolic and water extract of *Achyranthes* root, leaf, and stem was conducted to identify compounds with antiarthritic properties. Total 102 compounds were obtained, Out of 102 compounds, 27 compounds were found to have antirheumatic and antioxidant activities 9-Octadecenamide, (Z)- (Amide compound), Deoxyspergualin, Phytol (Diterpene), 1,2-15,16-Diepoxy Hexadecane, 17-Octadecynoic acid (Fatty acids), β -Sitosterol (sterol), Lupeol (triterpenoids), α -Amyrin (triterpene), β -Amyrin (triterpene), Taraxasterol, n-Hexadecanoic acid, L-(+)-Ascorbic acid 2,6-dihexadecanoate, 9,12-Octadecadienoic acid (Z,Z)-, Isopropyl linoleate, Isopropyl linoleate, 9-Octadecenamide, (Z)-, 2-Pyridineacetic acid, hexahydro-, 13-Docosenamamide, (Z)-, Octadecenamide, 12-hydroxy-, [R-(Z)]-.

Keywords: Rheumatoid arthritis, *Achyranthes*, Joint pain, inflammatory disorder.

INTRODUCTION

Rheumatoid arthritis is an inflammatory disorder that affects the joints of the body. Around 0.3-1% population is affected worldwide by this disorder. In developed countries women are more prone for this disease. In this disease joints are damaged to a large extent. There are many drugs available for the treatment of this disease. This disease mainly affects the joint cartilage which causes severe pain and function loss of the affected area. The main site of action of this disease is hip region (Male 9.6% and Female 13%) of 60 or above age group people. According to a study this disease will be the fourth disease by which people suffer disability by the 2020 (Gautam *et al* 2009). Joints are very versatile diarthrodial and homologous parts of the body. Typically a joint contains 1-Outer Membrane lined by Lamina propria and synovial layer.

1. Hyaluronan is transported into the synovial liquid by synovial cells.
2. Collateral ligaments-These are intracapsular and supports the joints
3. Articular Cartilage main function of this is to cover the articulating bone present inside the joints.
4. Subclonal bone gives the backing to the overlying cartilage and
5. The synovial fluid greases up and victuals the joint surface.

Synovial fluid main function is to provide lubrication and nourishment to the joints. Synovial fluid chemically is ultrafiltered plasma with hyaluron secreted by the synovium.

Glycosaminoglycan (GAG) is a vital component of both synovial fluid and articular ligament. The main function of this is to provide viscoelastic properties and to increase the compressive

resistance in articular ligament. HA is successful in limiting the action of plasma parts inside the synovial liquid

RA is a chronic inflammatory immune system disorder that causes a significant joint function loss (Breedveld *et al.*, 1997). It is a deformity of joints that causes loss of function. RA affects articular tissue also. This disease leads to substantial loss of mobility, pain, and joint destruction. It is a common disease having disease incidence in 3 and 4 decades of life with 3-5 higher precedence in females. RA most frequently affected joints of fingers and toes, wrists, knees, and ankles. Extra articular malfunctions can also occur and are often present in patients with severe diseases like ocular, pulmonary, hematologic, vascular, cardiac, neurologic, and mucosal tissues. Tetracycline antitoxins had been used to treat RA.

There is no great success has been achieved in getting a permanent cure for arthritis since the inflammation has started and articular damage begins (Huscisson *et al.*, 1982). But there are numerous therapies available for arthritis once it has occurred. These therapies include- Management of the immovability of joints by the physiotherapist and other measures. Prevention of infection by using antibiotics, food, and nutritional supplements help in the synthesis of proteoglycans, GAG, hyaluron and collagen which were vanished during the chronic attack.

Because of their side effects in the present time, people are running for herbal medicine and sometimes referred to as Herbalism or Botanical medicines. These herbal medicines are the oldest form of treatment from ancient times. Herbal plants have some therapeutically active chemicals substances which act on the diseased body part. In

the health care system of developed and developing countries, herbal medication gains extra attention. But their efficacy is very low because of their low absorptive value when administered orally. In the present time, herbal medicines are gaining popularity for various chronic diseases like Diabetes, arthritis, liver disease, cough remedies, memory loss, and many other diseases.

The main of this study was to evaluate the antirheumatic properties of *Achyranthes aspera*

Collection and Identification of plant material

Plant samples of *Achyranthes aspera*, was collected from the various regions of Kanpur district in 2016-2017 and taxonomically identified at the species level by Dr. Archana Srivastava, Botany department. The Voucher specimens also deposited in the Department of Botany, D.G.P.G. College, Kanpur. For *Achyranthes*, root, stem and leaf were used the fresh plant material is stored for anatomical studies and dried, using a mechanical grinder, a coarse powder made, and in a tight container stored for further use.

Organoleptic characteristics

Organoleptic characters like color, texture, and odor were recorded for each plant.

Phytochemical analysis

Preliminary chemical tests were carried out for crude powder and Methanolic/Aquas/Ethanol extracts to identify different Phyto-constituents (Harborne, 1973; Parekh and Chanda 2007c).

Metabolite profiling of plant materials

For *Achyranthes aspera*, ethanol and water were used, and for the remaining plants, methanol and water were used. 25 gm of powdered plant sample was measured and transferred into different beakers labeled according to solvents. 100ml of each solvent was measured and poured into the appropriate beaker, and stirred. Beakers were covered with foil paper to stop their evaporation. These were kept for 72 hours as such and then filtered and stored in bottles for phytochemical analysis

IISER Bhopal did GC-MS analysis. GCMS analysis was done by the SHIMADZU QP2010, an oven temperature from 50°C to 280°C at 4°C/min and held at this temperature for 5 min; inlet and interface temperatures were 250°C and 280°C, respectively. Helium used as carrier gas was (flow rate of 1.0 ml/min constant flow). 0.2 ml of sample injected under a split of 20:1. EIMS: electron energy, 70 eV. NIST database was used for the interpretation of GCMS mass spectra, which have more than sixty two thousand reported patterns. Obtained compounds were compared with available spectrum of known compounds present in NIST library.

RESULTS AND DISCUSSION

Organoleptic characters

Results of organoleptic characters observed for plant parts are mentioned below:

Table 1: Organoleptic characters of the used plant parts are as follows

SI No.	Name of the plant and Part	Nature	Colour	Odour	Taste
1	<i>Achyranthes</i> root	Smooth	Dark brown	Aromatic	Bitter
2	<i>Achyranthes</i> stem	Smooth	Dark brown	Aromatic	Bitter
3	<i>Achyranthes</i> leaf	Smooth	Dark brown	Aromatic	Bitter
4	<i>Cassia</i> leaf	Smooth	Dark green	Aromatic	Bitter
5	<i>Chenopodium</i> leaf	Smooth	Dark green	Aromatic	Bitter
6	<i>Chenopodium</i> stem	Smooth	Light green	Aromatic	Bitter
7	<i>Ricinus</i> root	Smooth	Light brown	Aromatic	Sweet

Table 2: Phytochemical analysis of selected plants

SI No.	Treatment	<i>Achyranthes</i> root (Water)	<i>Achyranthes</i> root (Ethanol)	<i>Achyranthes</i> stem (Water)	<i>Achyranthes</i> stem (Ethanol)	<i>Achyranthes</i> leaf (Water)	<i>Achyranthes</i> leaf (Ethanol)
1	Alkaloid detection						
	Mayer's Test	Positive	Positive	Positive	Positive	Positive	Positive
	Wagner's Test	Positive	Positive	Positive	Positive	Positive	Positive
	Carbohydrate detection						
	Molisch's	Negative	Negative	Positive	Positive	Positive	Positive

2	test						
	Benedict's test	Positive	Positive	Positive	Positive	Positive	Positive
	Fehling's test	Positive	Positive	Positive	Positive	Positive	Positive
Detection of glycosides							
3	Modified Borntrager's test	Negative	Negative	Positive	Positive	Negative	Negative
	Legal's test	Negative	Negative	Positive	Positive	Negative	Negative
Detection of phytosterols							
5	Salkowski's Test	Positive	Positive	Positive	Positive	Positive	Positive
	Liebermann Burchard's test	Positive	Positive	Positive	Positive	Positive	Positive
Detection of phenols							
6	Ferric chloride test	Positive	Positive	Positive	Positive	Positive	Positive
Detection of Tannins							
7	Gelatin test	Positive	Positive	Positive	Positive	Positive	Positive
Detection of flavonoids							
8	Alkaline Reagent Test	Positive	Positive	Positive	Positive	Positive	Positive
	Lead acetate test	Positive	Negative	Positive	Negative	Positive	Positive
Detection of proteins and amino acids							
9	Xanthoproteic Test	Positive	Positive	Positive	Positive	Positive	Negative
	Ninhydrin Test	Positive	Positive	Positive	Positive	Positive	Negative
Detection of diterpenes							
10	Copper Acetate Test	Positive	Positive	Positive	Positive	Positive	Positive

GC-MS analysis of the ethanolic and water extract of *Achyranthes* root, leaf, and stem was conducted to identify compounds with antiarthritic properties. Total 102 compounds were obtained, obtained compounds based on the molecular structure, mass, and calculated fragment ratio of resolved spectra with the mast spectra available from the library. Out of 102 compounds, 27 compounds were found to have antirheumatic and antioxidant activities 9-Octadecenamide, (Z)- (Amide compound), Deoxyspergualin, Phytol

(Diterpene), 1,2-15,16-Diepoxy Hexadecane, 17-Octadecynoic acid (Fatty acids), β -Sitosterol (sterol), Lupeol (triterpenoids), α -Amyrin (triterpene), β -Amyrin (triterpene), Taraxasterol, n-Hexadecanoic acid, 1-(+)-Ascorbic acid 2,6-dihexadecanoate, 9,12-Octadecadienoic acid (Z,Z)-, Isopropyl linoleate, Isopropyl linoleate, 9-Octadecenamide, (Z)-, 2-Pyridineacetic acid, hexahydro-, 13-Docosenamide, (Z), 9-Octadecenamide, 12-hydroxy-, [R-(Z)]-

Table 3: Compounds present in *Achyranthes* extract

<i>Achyranthes</i> leaf aqueous extract						
Sl. No.	Compound	Mol. Formula	Mol. Wt	Biological activity	RMF	MF
1	10-Heptadecene-8-ynoic acid, methyl ester, (E)-	C18H30O2;	278	Anticancer, Antitumor, Acidulant, Inhibit production of uric acid	679	666
2	4-Hydroxy-9-vinyl adamantane-2,6-dione	C12H14O3	206	Antimalarial, Antitumor, Anthelmintic	658	647
3	3,6,10,10-Tetramethyl-1-oxa-spiro[4.5]deca-3,6-dien-2-one	C13H18O2	206		745	643
6	Androstadiendione	C19H24O2	284		669	640
7	9-Octadecenamide, (Z)- (Amide compound),	C18H35NO	281	Antiinflammatory and Antibacterial	844;	828
8	9-Octadecenamide	C18H35NO	281		771	763
9	Deoxyspergualin	C17H37N7O3	387	Antiangiogenic, Immunosuppressant used in Rheumatism	752;	726
10	9-Octadecenamide, 12-hydroxy-, [R-(Z)]-	C18H35NO2	297		710	707
11	13-Docosenamide, (Z)-	C22H43NO	337	Antimicrobial	741	707
12	cis-11-Eicosenamide (Amide compound)	C20H39NO	309		705	705
<i>Achyranthes</i> leaf ethanol extract						
1	Phytol (Diterpene)	C20H40O	296	Antimicrobial, antioxidant, antiinflammatory, diuretic, chemopreventive, Antinociceptive	805	780
2	1,2-15,16-Diepoxy Hexadecane	C16H30O2	254	Antitumor, antiinflammatory	801	777
3	17-Octadecynoic acid (Fatty acids).	C18H32O2	280	Antioxidant, nematocide, pesticide	759	767
4	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C16H28O3	268		764	755
5	2-Methyl-Z,Z-3,13-octadecadienol	C19H36O	280	Anticancer,pesticide,herbicide,insecticide	781	746
6	3,7-Dimethyl-6-nonen-1-ol acetate	C13H24O2	212		764	744
7	γ -Sitosterol	C29H50O	414		902	898
8	β -Sitosterol (sterol)	C29H50O	414	Antimicrobial, anticancer, antiinflammatory, antiasthmatic, hepatoprotective, diuretic	846	799
9	Stigmast-7-en-3-ol,	C29H50O	414		867	742

	(3 β ,5 α ,24S)-					
10	Cholest-7-en-3-ol, 2,2-dimethyl-, (3 β ,5 α)-	C29H50O	414		788	721
11	Cholest-8(14)-en-3-ol, 4,4-dimethyl-, (3 β ,5 α)-	C29H50O	414		757	718
12	17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta[a]phenanthren-3-ol	C29H50O	414		717	714
13	Lupeol (triterpenoids)	C30H50O	426	Antimicrobial, Antiinflammatory, Anticancer	885	884
14	α -Amyrin (triterpene)	C30H50O	426	Antimicrobial, Antiinflammatory	814	790
Achyranthes root extract						
	Compounds	Mol. Formula	Mol. Wt.	Biological activity	RMF	MF
2	D-Glucose, 4-O- α -D-glucopyranosyl-	C12H22O11	342	No activity is reported	744	737
3	Desulphosinigrin	C10H17NO6S	279	No activity is reported	738	728
4	d-Glycero-d-galacto-heptose	C7H14O7	210	No activity is reported	737	726
5	d-Glycero-d-ido-heptose	C7H14O7	210	No activity is reported	741	721
6	Lactose	C12H22O11	342	No activity is reported	745	713
Achyranthes root ethanol extract						
1	Hexadecanoic acid, methyl ester	C17H34O2	270	Antibacterial and antifungal	922	918
2	Pentadecanoic acid, 14-methyl-, methyl ester fatty ester	C17H34O2	270	No activity is reported	869	854
3	Hexadecanoic acid, 2-methyl-	C17H34O2	270		826	801
4	Pentadecanoic acid, 13-methyl-, methyl ester	C17H34O2	270		781	780
5	Hexadecanoic acid, 15-methyl-, methyl ester	C18H36O2	284		848	765
6	Pentadecanoic acid, methyl ester	C16H32O2	256		845	765
7	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	C6H6O3	126		805	707
8	6-Acetyl- β -d-mannose	C8H14O7	222		723	673
9	4-Octen-3-one, 6-ethyl-7-hydroxy-	C10H18O2	170		729	653
10	Hydrazinecarboxamide, 2-(2,6-dimethyl cyclohexylidene)-	C9H17N3O	183		666	645
11	4-Mercaptophenol	C6H6OS	126		700	638
12	Tetrahydropyran-4-ol, 2,2-dimethyl-4-[[[(thiophen-2-ylmethyl)amino]methyl]-	C13H21NO2S	255		698	636
13	n-Hexadecanoic acid	C16H32O2	256	Antioxidant, antiinfl	884	871

	,palmitic acid			ammatory,nematicidal,		
14	l-(+)-Ascorbic acid 2,6-dihexadecanoate	C38H68O8	652	Antiallergic,antianemic,antibacterial,anticancer,anticataract,anticoagulant,antidiabetic,antiinflammatory,antigastric,antimalarial,antihepatic,antihypertensive,antiplague,antitumor,antitubercular,neuroprotective	833	833
15	Palmitic anhydride	C32H62O3	494		785	783
16	Pentadecanoic acid Lubricants, Adhesive agents,fatty acid	C15H30O2	242	Lubricant,adhesive agents	838	767
17	Isopropyl Palmitate	C19H38O2	298		766	759
18	Tetradecanoic acid	C14H28O2	228	Larvicidal and repellent activity	832	749
19	14,17-Octadecadienoic acid, methyl ester	C19H34O2	294		876	843
20	Methyl 12,15-octadecadienoate	C19H34O2	294		863	828
21	Methyl 11,14-octadecadienoate	C19H34O2	294		867	823
22	11,14-Octadecadienoic acid, methyl ester	C19H34O2	294		861	822
23	12,15-Octadecadienoic acid, methyl ester	C19H34O2	294		859	822
24	9,12-Octadecadienoic acid, methyl ester, (E,E)-	C19H34O2	294		825	818
25	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	,antiarthritic,	843	835
26	Isopropyl linoleate	C21H38O2	322	Antioxidant,antiinflammatory	830	797
27	Octadecynoic acid	C18H32O2	280	antihypertensive	830	797
28	i-Propyl 9,12-octadecadienoate	C21H38O2	322		812	797
29	2-Methyl-Z,Z-3,13-octadecadienol anticancer	C19H36O	280	Anticancer	837	783
30	12-Methyl-E,E-2,13-octadecadien-1-ol(E)-Lenoic acid	C19H36O	280	Antihistaminic, antibacterial,allergenic, antisalmonella, anticonvulsant, antiseptic	814	782
Achyranthes stem water extract						
1	α -Pyrrolidone, 5-[3-hydroxybutyl]-	C8H15NO2	157		749	733
2	2-Cyclohexyl Piperidine	C11H21N	167		759	722
3	Bicyclo[2.2.1]heptan-2-ol, 3-amino-1,7,7-trimethyl-, (endo,endo)-	C10H19NO	169		751	695

4	Pyrrolidine, 1,2-dedihydro-5-[3-hydroxy-1-butyl]-2-methylthio-	C9H17NOS	187		707	690
5	2-Pyridineacetic acid, hexahydro-,	C7H13NO2	143	Lowers uric acid production,acidulant, arachidonic acid inhibitor	826	676
6	α -Pyrrolidone, 5-[3-bromo-1-butyl]-	C8H14BrNO	219		737	674
7	9-Octadecenamide, (Z)-	C18H35NO	281	Anti-inflammatory,antibacterial	824	778
8	Isopropyl linoleate	C18H35NO	281	Anti inflammatory,antibacterial	816	776
9	Deoxyspergualin	C17H37N7O3	387	Immunosuppressive	801	770
10	9-Octadecenamide, 12-hydroxy-, [R-(Z)]-	C18H35NO2	297	aryl hydrocarbon hydroxylase inhibitor, 17-beta hydroxysteroid dehydrogenase inhibitor, 5-alpha reductase inhibitor	750	745
11	cis-11-Eicosenamide	C20H39NO	309	4.59%	736	735
12	6-Octadecenoic acid	C18H34O2	282	Acidifier	735	691
Achyranthus stem ethanol extract						
1	Hexadecanoic acid,	C17H34O2	270	Lowers the uric acid production	895	889
2	Pentadecanoic acid, 14-methyl-, methyl ester	C17H34O2	270	increase aromatic amino acid decarboxylase activity,acidifier,acidulant,methyl donor	833	826
3	Hexadecanoic acid, 2-methyl-	C17H34O2;	270	acidulant,inhibit production of uric acid	801	770
4	Pentadecanoic acid, 1,3-methyl-, methyl ester	C17H34O2	270	urine acidifier,urinary acidulant, methyl donor	721	719
5	Hexadecanoic acid, 15-methyl-, methyl ester,	C18H36O2;	284	inhibit production of uric acid,methyl donor,acidifier,arachidonic acid inhibitor	797	717
6	Pentadecanoic acid, methyl ester	C16H32O2	256	methyl guanidine inhibitor,acidifier,acidulant	793	705
7	n-Hexadecanoic acid,	C16H32O2	256	acidifier,inhibit production of uric acid,antitumor,increase NK cell activity	847	824
8	l-(+)-Ascorbic acid 2,6-	C38H68O8	652		786	782

	dihexadecanoate					
9	Isopropyl Palmitate	C19H38O2	298		735	723
10	Pentadecanoic acid	C15H30O2;	242		787	719
11	Palmitic anhydride	C32H62O3	494		731	717
12	i-Propyl 14-methyl-pentadecanoate	C19H38O2	298		707	725
13	9,12-Octadecadienoic acid, methyl ester, (E,E)-	C19H34O2	294		861	854
14	7,10-Octadecadienoic acid, methyl ester,	C19H34O2;	294	arachidonic acid inhibitor,acidulant	867	852
15	10,13-Octadecadienoic acid, methyl ester,	C19H34O2	294	acidifier,acidulant,inhibit production of uric acid	849	845
16	14,17-Octadecadienoic acid, methyl ester,	C19H34O2	294	catechol-o-methyltransferase inhibitor	882	843
17	Methyl 12,15-octadecadienoate,	C19H34O2	294	,catechol-o-methyltransferase inhibitor	882	843
18	8,11-Octadecadienoic acid, methyl ester	C19H34O2	294		848	843
19	9-Octadecenamide, (Z)-,	C18H35NO	281		814	802
20	9-Octadecenamide	C18H35NO	281		696	687
21	13-Docosenamide, (Z)- #	C18H35NO2	337	Anti-inflammatory, intestinal Albendazole monooxygenase inhibitor Antitoxic	710	679
22	9-Octadecenamide, 12-hydroxy-, [R-(Z)]-	C18H35NO2	297	Antiinflammatory activity and antibacterial activity	661	655
23	cis-11-Eicosenamide	C20H39NO	309		650	650
24	Deoxyspergualin,	C17H37N7O3	387	Immunosuppressive agent, Antiangiogenic action	664	633

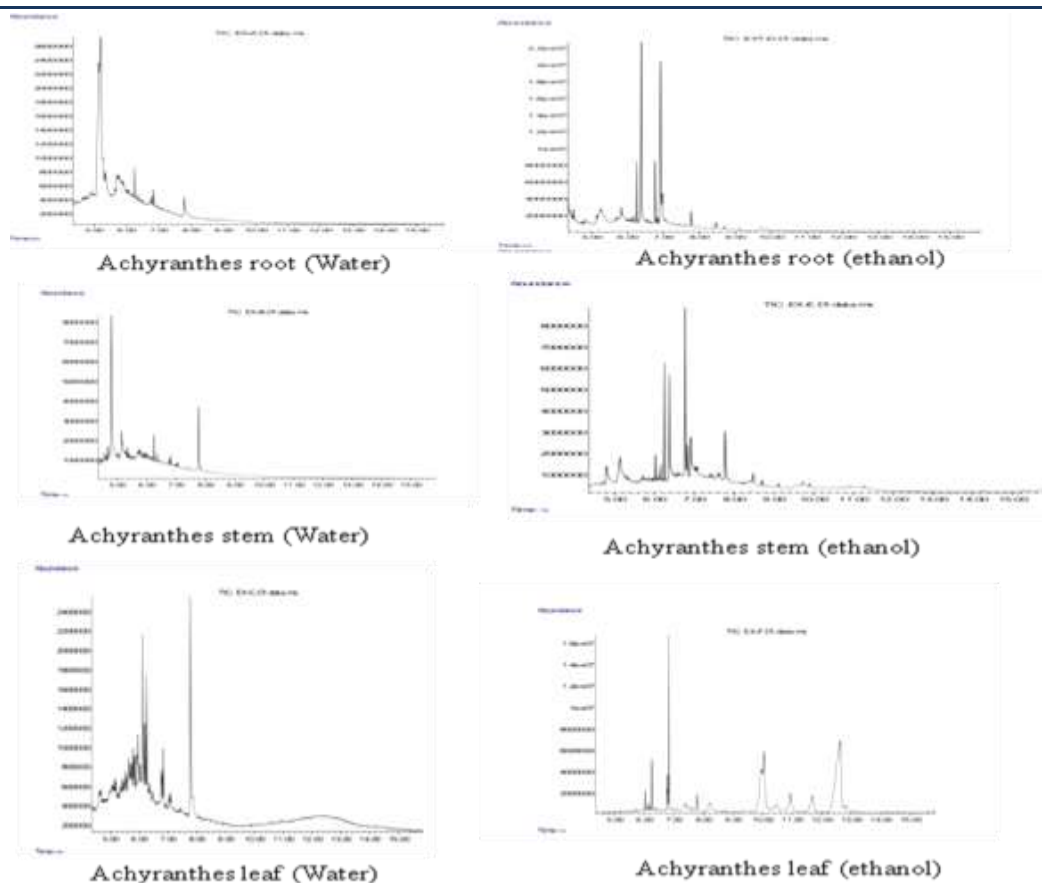


Fig.1. GCMS Chromatogram of Achyranthes plants metabolites

Metabolic profiling of plant species was done by Gas chromatography. The identified compounds have many biological properties. GC-MS analysis of the phytoconstituents in plants gave a clear picture of the plants' pharmaceutical value for arthritis treatment. This type of study helps understand the nature of plants and identify their antirheumatic potential. Flavonoids are well known for their antibacterial, anti-inflammatory, anti-allergic, and antiviral activity (Alan and Miller, 1996). Alkaloids have also been known for their pharmacological effects as medication and recreational drugs (Roger and Wink, 1998). Tannins combine with phenols and form a polyphenolic group known for their antioxidant, anti-inflammatory, and antimicrobial activities. As suggested by Mehta *et al.* 2012 terpenoids, also have been found to possess protective activities against arthritis. The phenolic compounds are the most commonly found secondary metabolite. Phenolic compounds have a great antioxidant potential which is attributed to their redox behavior.

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