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Head Space GC/MS Analysis of Volatile Constituents of *Trichilea connaroides* Wight and Arn. Extracts and their *in vitro* Anti-Plasmodium Activity Against *Plasmodium falciparum* Isolates

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ABSTRACT

Head space GC-MS analysis ethyl acetate extracts of leaves, bark, root and pericarps of *Trichilia connnaroides* Wight and Arn. Revealed the presence of over 45 compounds of which 22.06, 97.24, 46.42 and 58.27% of the total volatiles from extracts of leaves bark, root and pericarps were identified, respectively. The volatile constituents of bark extract were rich in sesquiterpenoides. Copaene (24.71%), azulene (17.47%), α-cubebene (14.98%), β-cadinene (12.58%), α-bergamotene (4.96%) and ylangene (5.50%) were the major constituents of the total volatiles of the extract. The identified constituents in other extract were 22.06% in leaves extract, 46.42% in root extract and 58.26% in pericarp extract, respectively. *In vitro* antiplasmodium activity of five extract were tested against K 1 strain of *Plasmodium falciparum* isolates. All the extract showed antiplasmodial activity. Relatively high activity was observed in dichloromethane extract.

Key words: *Trichilia connnaroides, Plasmodium falciparum,* copaene, azulene, antiplasmodium activity

INTRODUCTION

Preparations from leaves, seeds, stem, bark and roots of many plants belonging to the family Meliaceae have been widely used in traditional medicine. Antiviral, antihelmintic, antitumoral, anti-inflammatory and antirheumatic activities of the plant family Meliaceae have been described Bhakuni *et al.* (1969), Fujiwara *et al.* (1982), Patel (1986), Andrei *et al.* (1990), Bray *et al.* (1990) and Coulombie *et al.* (1992). The family Meliaceae is known to be a rich source of limonoids, which possess interesting biological activities against insects such as antifeeding, deterrent and inhibitors of ecdyasis (Champagne *et al.,* 1992).

Malaria is one of the most prevalent diseases in the world. It affects more than 500 million each year, mostly from sub-Saharan Africa and Asia causes about 2.3 million deaths a year (World Health Organization, 1996). The problems of the resistance of the vector mosquitoes to insecticides and to the parasites to most of the commercially available antimalarials are a serious problem (Wernsdorfer and Trigg, 1988). Members of the Meliaceae have been used for generations in Africa, India and tropical America to treat malaria. In tropical America *Cedrela odorata, Carapa*

quianensis and *Swietenia mahagoni* have been used while in Africa and India the 'Neem' tree or *Azadirachta indica* is used (Mac-Kinnon *et al.,* 1997). Some other plant species belonging to the Meliaceae family viz. *Khaya grantifoliola*, *Entendrophragma utile* and *Morinda lucida* are also widely used as antimalarials or antipyretics in traditional medicine (Bray *et al.,* 1990; Bickii *et al.,* 2000; Obih *et al.,* 1985; Weenen *et al.,* 1990). These plants are sources of poly oxygenated terpenoids called limonoids, biosynthetically related to quassinoids whose antiplasmodial and cytotoxic activities have been demonstrated (Bray *et al.,* 1990; Connolly, 1983).

Trichilia connaroides (Wight and Arn.) Bentv. Syn. *Heynea trijuga* Roxb. is a tall tree widely distributed in the South and East of Asia, such as India, Indonesia and South China (Chen *et al.,* 2007). It has been reported that of *T. Connaroides* posses analgesic and anti-inflammatory activity (Purnima *et al.,* 2006). Larvicidal activity against *Peridroma saucia* and *Spodoptera litura* have also been reported (Xio *et al.,* 1994). We also have earlier reported hypotensive activity of *T. connaroides* extracts in rats (Agarwal *et al.,* 2006) and growth regulatory activity of *T. connaroides* leaf extracts against the Bihar hairy caterpillar *Spilosoma oblique* (Lepidoptera: Arctiidae) (Tandon *et al.,* 2009).

The present study is on investigation of head-space GC/MS analysis of volatiles from plant parts and *in vitro* antimalarial activity of different extracts of *T. connaroides* Wight. and Arn. fruit pericarps against *P. falciparum* isolates.

MATERIALS AND METHODS

Plant collection: The plant *Trichilia connnaroides* Wight and Arn. was collected from Ranibagh, District Nainital, Uttarakhand, India in the month of October, 2008. Identification of this plant was confirmed by Prof Y.P.S. Pangty, Professor of botany and plant taxonomist, Kumaon University, Nainital. The herbaria was deposited and maintained in Department of Chemistry, G.B. Pant University of Ag. and Tech., Pantnagar.

Preparation of extracts: Fresh leaves, bark, root and seeds of *Trichilia connaroides* were collected, seed coats (pericarps) removed separately, shade dried, powdered and extracted with diethyl ether and the solvent was removed under vacuum. Seed coats (paricarps) were also subjected to extraction using cold extraction process in a percolator first in petroleum ether for three days. The process was repeated for three times. Same process was followed with diethyl ether,dichloromethane, chloroform and methanol. The solvents were evaporated using thin film vacuum ratatory evaporator. The extracts were kept in refrigerator for further use. The yields are presented in Table 1.

Gc-ms analysis (head space analysis): GC-MS analysis of the diethyl ether extracts of leaves, bark, root and pericarps were performed on a Agilent 19091s-433 with MS detector, using a HP-

Table 1: *In vitro* antiplasmodial activity of pericarp extracts of *T. connaroides* against *P. falciparum* isolates

Extracts w/w yield (%)	IC_{50} (μg mL^{-1})
Petroleum ether (5.2)	14.79
Diethyl ether (1.2)	9.44
Dichloromethane (4.8)	6.92
Chloroform (3.2)	9.33
Methanol (3.2)	21.93
Chloroquine	0.051

Data shown are values from two replicate experiments

SMS capillary column (30 m x 0.25 mm id) with a temperature program from 50 to 280°C at 1.5°C min^{-1} and finally held at 280°C. Helium was the carrier gas (flow rate 1.1 mL min^{-1}), ion source temperature was 230°C and the ion inlet temperature 220°C. The MS were recorded under EI conditions (70 ev) with a split less mode. Sample components were identified by comparing their mass spectra with those in the NIST/Wiley Library and by comparison with literature data and GC retention indices (Adams, 1995).

One gram of sample is taken in 20 mL headspace vial. Headspace septum was obesotead and these vapours were injected in GC equipped with MSD.

- **Zone temp: Vial temperature:** 120°C
- **Loop temperature:** 130°C
- **Transfer line temp:** 150°C
- **Event time: Vial equilibration time:** 15.0 min
- **Pressurizing time:** 0.20 min
- **Loop fill time:** 0.05 min
- **Loop equilibration time:** 0.05 min
- **Injection time:** 10.0 min

Drug sensitivity assays: Plant extracts were dissolved in dimethyl sulfoxide (DMSO) to obtain desired concentrations and were screened for antiplasmodial activity against K1 strain of *Plasmodium falciparum* isolates.

***In vitro* antiplasmodium activity:** *In vitro* drug sensitivity of extract was carried out at National Institute of Malaria Research, Delhi, India as per procedure described by Trager and Jensen (1976). Chloroquine sensitive *Plasmodium falciparum* FSG strain derived from an Indian patients of Uttar Pradesh was used for the study using *in vitro* candle-jar method as described by Trager and Jensen (1976). Culture was maintained in A^+ erythrocytes using RPMI 1640 medium supplemented with AB Rh +ve human serum (10%), sodium bicarbonate (0.2%), HEPES buffer (25 mM) and gentamycin (50 μg mL^{-1}). The culture was treated with selected concentrations (50, 10, 5, 2.5, 1.25 μg $well^{-1}$) of *T. connaroides* extracts. After 72 h of incubation, blood smears were prepared and stained with Giemsa strain. Percentage maturation of schizonts against control was recorded. Chloroquine was used as a standard drug. The inhibitory concentration values which kills 50% of the parasites (IC_{50}) were considered for anti-plasmodial activity.

RESULTS AND DISCUSSION

Chemical composition of diethyl ether extract of leaves, bark, root and pericarps: The results of Head Space analysis of diethyl ether extracts of leaves, bark, root and seed pericarp are recorded in Table 2. Bark volatiles are rich in sesquiterpenoides. The major components identified were azulene (17.47%), cubebene (14.98%), ylengene (5.5%) and copaene (24.71%) of the total volatiles. The total volatile volatiles identified in other extracts were 22.06% in leaves extract, 46.42% in root extract and 58.27% in pericarp extract respectively. The major volatile compounds identified in leaf extract were azulene (8.62%), caryophyllene (1.76%), copaene (1.10%) and β-bourbonene (1.02%) of the total volatiles. The major volatiles identified in root extract were azulene(10.62%), 2-methyl-2-bornene(2.36%), α-bergamotene (5.03%), β-cedrene(3.65%) and β-chamigrene(10.2%)of the total volatile of root extract. Caryophyllene (14.13%), cis- calamenene

Table 2: Chemical composition of diethyl ether extracts (%) of *Trichilia connaroides*

Compounds	Leaves	Bark	Root	Pericarp
Heminellitene	-	-	2.75	-
2-methyl-2-bornene	-	-	2.36	-
Eucalyptol	-	-	-	1.53
cis-ocimene	-	-	-	2.46
Azulene	8.62	17.47	10.62	2.13
Cuminol	-	-	-	1.56
β-cyclocitral	0.47	-	-	-
α-cubebene	0.63	14.98	0.63	2.48
Ylangene	0.41	5.50	0.66	0.52
α-bourbonene	0.84	-	-	0.48
Copaene	1.10	24.71	1.93	4.92
β-bourbonene	1.02	-	-	-
β-cubebene	-	1.40	-	0.98
Germacrene D	-	2.79	-	1.14
Cyclosativene	-	0.38	0.38	2.16
Isoledene	-	3.41	0.78	-
α-bergamotene	-	4.96	5.03	-
α-gurjunene	-	-	0.23	1.08
β-cedrene	-	-	3.65	-
β-caryophyllene	1.76	2.87	-	14.13
(+)-*epi*-bicyclosesquiphellandrene	-	-	-	0.99
γ-cadinene	0.45	-	-	0.52
δ-selinene	-	-	0.49	-
(-)-isoledene	-	-	-	2.15
α-santalene	-	-	1.30	-
α-caryophyllene	0.57	-	-	3.01
Aromadendrene	-	-	-	0.66
δ-cadinene	0.72	12.58	-	0.79
α-longipinene	-	-	0.53	-
β-chamigrene	-	-	10.20	-
γ-murollene	0.79	-	0.97	0.21
α-selinene	-	2.24	-	-
β-patcholene	0.41	-	-	1.20
β-elememe	-	-	-	0.63
α-farnesene	0.98	-	-	-
β-bisabolene	-	1.13	-	-
Calamenene	-	-	2.36	6.34
δ-selinene	-	-	-	0.93
β-gurjunene	-	-	1.55	-
Cadiene-1,4-diene	-	2.82	-	3.10
α-elemene	-	-	-	0.80
α-muurolene	-	-	-	1.58
Caffeine	2.00	-	-	-
Eicosane	0.76	-	-	-
Palmitic acid	0.53	-	-	-
Total	22.06	97.24	46.42	58.27

(6.34%), copaene (4.92%), cadiene-1,4-diene (3.10%), α-caryophyllene (3.01%) were the major components present in the total volatiles of pericrap extract. The detailed compositions of the volatiles are recorded in Table 2.

***In vitro* antiplasmodium activity different extracts of pericarps:** Five crude organic extracts obtained from *T. connaroides* pericarps were tested *in vitro* against *P. falciparum*. Two extracts (dicloromethane and chloroform) of seed pericarp of *T. connaroides* were effective against *Plasmodium falciparum* K1 strain. Other extracts viz., petroleum ether, diethylether and methanol showed weak antiplasmodial activity against *P. falciparum* K1 strain as comparison to the standard drug chloroquine (IC_{50} = 0.051μg mL^{-1}). IC_{50} values of extracts were between 6 and 22 μg mL^{-1} Table 1.

Earlier, Rochanakij *et al.* (1985) identified nimbolide as the active antimalarial principle of the Neem tree. Gedunin and its dihydro derivative were also found to be active *in vitro against P. falciparum* (Mac-Kinnon *et al.,* 1997). Limonoids, which exhibit in vitro antimalarial activities, have been reported from *Cedrela odorata* (Bray *et al.,* 1990), *Khaya senegalensis* (Khalid *et al.,* 1998) and *Khaya grantifoliola* (Bickii *et al.,* 2000). Two limonoids, trichirubine A and B have been isolated from *T. rubescens* with significant antimalarial activity (Krief *et al.,* 2004). Their antimalarial activities may be related to the presence of reactive groups on ring A like the carbonyl group at C-3 and unsaturation in C-1/C-2 positions. The limonoid derivatives 7-deacetylgedunin and 7-deacetyl-7-oxogedunin isolated from the roots of *Pseudocedrela kotschyi* have been reported to display moderate antimalarial activity (Hay *et al.,* 2007).

Trichilia connaroides is a rich source of triterpenoids, limonoids and a steroid have been characterized from the leaves, flowers, roots, or pericarps of *Trichilia connaroides* (Puroshothaman *et al.,* 1983, 1987; Venkatanarsimhan *et al.,* 1990; Inada *et al.,* 1994; Zhang *et al.,* 2003; Wang *et al.,* 2008; Geng *et al.,* 2009). Therefore the antiplasmodial activity of *T. connaroides* pericarp extracts could be due to limonoids.

CONCLUSIONS

The volatiles from leaves, bark, roots and fruit preicarp of *Trichilia connaroides* are being reported for the first time. The extracts of the plant parts posses antiplasmodial activity. The results are of pharmaceutical interest for further drug discovery programmes.

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REFERENCES

Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Co., Carol Stream, IL., USA., pp: 69-351.

Agarwal, G., S.K. Hore, O. Prakash and A.K. Pant, 2006. Hypotensive activity of *T. connaroides* extracts in rats. J. Vet. Pharmcol. Toxicol., 5: 72-73.

Andrei, G.M., F.C. Coulombie, M.C. Courreges, R.A. de Torres and C.E. Coto, 1990. Melicine, an antiviral compound from *Melia azedarach* L., inhibits interferon production. J. Interferon Res., 10: 469-475.

Bhakuni, D.S., M.L. Dhar, M.M. Dhar, B.W. Dhawan and B.N. Mehratra, 1969. Screening of plants for biological activity. Part II. Indian J. Exp. Biol., 7: 250-262.

Bickii, J.N., J. Njifutie, J.A. Foyere, L.K. Basco and P.J. Ringwald, 2000. *In vitro* antimalarial activity of lomonoids from *Khaya grantifolia* C.D.C (Meliaceae). Ethnopharmacology, 69: 27-33.

Bray, D.H., D.C. Warhurst, J.D. Connolly, M.J. O'Neill and J.D. Phillipson, 1990. Plants as sources of antimalarial drugs. Part 7. Activity of some species of meliaceae plants and their constituent limonoids. Phytotherapy Res., 4: 29-35.

Champagne, D.E., O. Koul, M.B. Isman, G.G.E. Scudder and G.H.N. Towers, 1992. Biological activity of limonoids from the Rutales. Phytochemistrym, 31: 377-394.

Chen, Y.Y., X.N. Wang, C.Q. Fan, S. Yin and J.M. Yue, 2007. Swiemahogins A and B, two novel limonoids from *Swietenia mahogany*. Tetrahedron Lett., 48: 7480-7484.

Connolly, D.L., 1983. Chemistry of Limonoids of the Meliaceae and Cneoraceae. In: Chemistry and Chemical Taxonomy of the Rutales, Waterman, P.G. and M.F. Grundon (Eds.). Academic Press, London.

Coulombie, F.C., G.M. Andrei, R.P. Laguens, R.A. de Torres and C.E. Coto, 1992. Partially purified leaf extracts of *Melia azedarach* L. inhibit Tacaribe virus growth in neonatal mice. Phytotherapy Res., 6: 15-19.

Fujiwara, T., T. Takeda, Y. Ogihara, M. Shimizu, T. Nomura and T. Tomita, 1982. Studies of the structure of polysaccharides from the bark of *Melia azadirachta*. Chem. Pharm. Bull., 30: 4025-4030.

Geng, Z.L., X. Fang, Y.T. Di, Q. Zhang, Y. Zeng, Y.M. Shen and X.J. Hao, 2009. Trichilin B, a novel limonoid with highly rearranged ring system from *Trichilia connaroides*. Tedrtraheon Lett., 50: 2132-2134.

Hay, A.E., J.R. Ioset, K.M. Ahua and D. Diallo, R. Brun and K.J. Hostettmann, 2007. Limonoides orthoacetates and antiprotozoal compounds from the roots of Pseudocedrela katschyi. J. Nat. Prod., 70: 9-13.

Inada, A., M. Konishi, H. Murata and T. Nakanishi, 1994. Structure of new limilnoid and a new triterpenoid derivative from pericarp of *Trichillia connaroides*. J. Nat. Prod., 57: 1446-1449.

Khahd, S.A., G.M. Friedrichsen, A. Kharazmi, G.T. Theander, C.E. Olsen and C.S. Brogger, 1998. Liminoids from *Khaya senegalensis*. Phytochemistry, 49: 1769-1772.

Krief, S., M.T. Martin, P. Grellier, J. Kasenene and T. Sevenet, 2004. Novel antimalerial compounds isolated in a survey of self medicative behaviour of wild chimpanzees in Uganda. Antimicrob. Agents Chemother., 48: 3196-3199.

Mac-Kinnon, S., T. Durst, J.T. Arnason, C. Angerhofer and J. Pezutto *et al.*, 1997. Antimalarial activity of tropical Meliaceae extracts and Gedunin derivatives. J. Nat. Prod., 60: 336-341.

Obih, P.O., J.M. Makinde and J. Laoye, 1985. Investigation of various extracts of *Morinda lucida* for antimalarial actionsm on *Plasmodium berghei* in mice. Afr. J. Med. Sci., 14: 45-49.

Patel, N.G., 1986. Indian Traditional Medicine. In: Folk Medicine: The Art and Science, Steiner, R.P. (Ed.). American Chemical Socity, Washinggton DC., pp: 57.

Purnima, A., G.S. Prasanna and V. Mathuram, 2006. Analgesic and antiinflammatory activity of the chloroform extract of *Trichilia connaroides* (W. and A.) Bentilizen. Indian J. Pharmaceutical Sci., 68: 231-233.

Puroshothaman, K.K., A. Sarada and V. Mathuram, 1983. Structure of Heynic acid: A new triterpene acid from *Heynea trijuga* roxb. Ind. J. Chem., 22B: 820-821.

Puroshothaman, K.K., M. Sarada, Venkatanarsimhan, A. Sarada, J.D. Connolly and D.S. Rycroft, 1987. Trigugins A and B, tetranorterpenoids with a novel rearranged carbon skeleton from *Heynea trigjuga* (Meliaceae). Can. J. Chem., 65: 35-37.

Rochanakij, S., Y. Thebtaranonth, C.H. Yenjal and Y. Yuthavong, 1985. Nimbolide, a constituent of *Azadirachta indica* inhibits plasmodium falciparum in culture Southeast Asian. J. Trop. Med. Public Health, 16: 66-72.

Tandon, S., A.K. Mittal and A.K. Pant, 2009. Growth regulatory activity of *T. connaroides* (syn. *Heynea trijuga*) leaf extracts against the Bihar hairy caterpillar *Spilosoma oblique* (Lepidoptera: Arctiidae). Int. J. Trop. Insect Sci., 29: 180-184.

Trager, W. and J. Jensen, 1976. Human malaria parasites in continuous culture. Sciences, 193: 673-675.

Venkatanarsimhan, M., A.B. Kundu and A. Patra, 1990. Isolation and characterization of trijugin-B acetate from *Heynea trijuga* Roxb. Indian J. Chem., 29B: 970-970.

Wang, X.N., C.Q. Fan, S. Yin, L.S. Gan and J.M. Yue, 2008. Structural elucidation of limonoids and steroids from *Trichilia connaroides*. Phytochemistry, 69: 1319-1327.

Weenen, H., M.H. Nkunya, D.H. Bray, L.B. Mwasumbi, L.S. Kinabo and V.A. Kilimali, 1990. Antimalarial activity of Tanzanian medicinal plants. Planta Medica, 56: 368-370.

Wernsdorfer, W.H. and P.I. Trigg, 1988. Recent Progress of Malaria Research: Chemotherapy. In: Malaria Principle and Practice of Malariology, Wernsdorfer, W.H. and S.I. McGregor (Eds.). Churchill Livingstone, London, pp: 1569-1674.

World Health Organization, 1996. Malaria distribution. A Weekly Epidemiol. Record, 71: 17-24.

Xio, Y.S., M.B. Isman, P. Gunning, S. MacKinnon and J.T. Arnason *et al.*, 1994. Biological activity of extracts of Trichilia species and the limonoid hirtin against lepidopteran larvae. Biochem. Syst. Ecol., 22: 129-136.

Zhang, H.P., S.H. Wu, Y.M. Shen, Y.B. Ma, D.G. Wu, S.H. Qi and X.D. Luo, 2003. Apentanortriterpenoid with a novel carbon skeleton and new pregnane from atrichilia connaroides. Can. J. Chem., 81: 253-257.