Summary

The present study was hypothesized to investigate protective effect of rhizome of *Hedychium spicatum var. acuminatum* and galls of *Pistacia integerrima* J. L. Stewart ex Brandis against cough and asthmatic disorder. The fresh rhizomes of *H. spicatum* in wild from Habban, Sirmour, Himachal Pradesh, India and leaf galls of *P. integerrima* from both wild and cultivated trees from Narag, Sirmour, Himachal Pradesh were collected. They were taxonomically identified and authenticated and a voucher specimen were also deposited in UHF-Herbarium with field book number 13457 and 13507 respectively. Standardization of the selected plant material was done by pharmacognostical and physiochemical evaluation. Pharmacognostical evaluation revealed macroscopic and microscopic characteristics inherited by rhizome of *H. spicatum* and galls of *P. integerrima* respectively. Physicochemical evaluations on rhizomes of *H. spicatum* and leaf galls of *P. integerrima* were aimed to meet various physicochemical standards such as foreign matter, ash values (total ash, water-soluble ash and acid-insoluble ash), loss on drying, extractive values (pet ether soluble, ethanol soluble and water soluble) comparable to the monograph of selected plant material and to ensure their safety, efficacy and quality. All physicochemical standards were conducted within limits in accordance with the monograph of these two plants as per The Ayurvedic Pharmacopoeia of India. The phytochemical evaluation of the extracts prepared and phytochemical screening give a gross idea about the presence of different plant secondary metabolites in given plant by their qualitative tests. Both the plants had finding like low polar constituents showed presence as fixed oils and volatile oils in pet ether extract whereas chloroform extract showed the presence of fixed oils, volatile oils, flavonoids and phytosterols, moderate polar solvent like ethyl acetate shows the presence of phytosterols, phenolic compounds, and flavonoids, polar solvents like methanol and water showed the presence of alkaloids, glycosides, flavonoids, phenols, tannins, triterpenoids, saponins, carbohydrates and proteins. Isolation and physicochemical characterization of volatile oil was carried out for both the selected plants the finding were that the volatile oil of rhizomes of *H. spicatum* was having colorless to pale yellow color with agreeable aromatic, pleasant and camphor like odor with % age yield 1.80±0.012 % v/w and volatile oil of galls of *P. integerrima* was having colorless to pale white color with strong aromatic, pleasant and turpentine like odor.
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odor and % age yield 1.50±0.015% v/w. GC-MS of oil of the volatile oil rhizomes of *H. spicatum* (SUPYA-HA) confirmed the presence of terpenoids compounds: Alpha-Pinene, Beta-Pinene, 1,8 –Cineole, Linalool, Alpha Terpineol, Delta Cadinene, Hedycaryl, Germacrene D-4-ol, Juniper Camphor, 10-epi Gama Eudesmol, T-muurolol, Beta-Eudersmol, Alpha Cadinol and 1(5)-Guaien-11-ol. GC-MS of the Volatile oil of galls *P. integerrima* (SUPYA-PC) confirmed also the presence of terpenoids.: 2-Thujene, (1R)-(+) Alpha-Pinene, Camphene, p-Mentha-1(7),2-diene, Beta-Pinene, Myrcene, (3-Carene, 4-Carene, O-Cymene, (+)-Dipentene, Gamma Terpinene, Terpinolene, (-)-Terpinen-4-ol, (-)-Alpha-Terpineol and Beta-Caryophyllene. Whereas the GC-MS of pet ether extract of both rhizomes of *H. spicatum* and galls *P. integerrima* which were coded as SUPYA-HA-PE and SUPYA-PC-PE respectively. SUPYA-HA-PE showed presence of number of low polar volatile compounds mainly coumarins, terpenoids and fatty acids. Total 85 compounds were identified out of these 27 compounds having amount % age more than 1% are Terpenoids: (1R)-(+) Alpha-Pinene (2.32%), Beta-Pinene (4.72%), 1,8-Cineol (8.47%), Beta-Linalool (1.70%), L-4-Terpineol (1.25%), Levo(-)-alpha-Terpineol (1.99%), Beta-Caryophyllene (1.76%), Alpha-Caryophyllene (1.37%), cis-β-Farnesene (2.33%), Cubedol (1.19%), α-Murolene (1.26%), Gamma-Cadinene (3.06%), (+) Delta-Cadinene (3.29%), Hedycaryl (9.93%), Nerolidol (1.28%), 1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene (3.64%), (-)-Beta-Caryophyllene oxide (1.69%), Globulol (1.73%), (-)-Cubenol (1.48%), 10-epi Gama Eudesmol (2.81%), Gamma eudesmol (3.05%), α-Cadinol (10.16%), (-)-Isoaromadendrene epoxide (1.03%), 2-methyl-2-(3-methyl-2-oxo-butyl) cyclohexanone (1.60%), Rosifoliol (1.09%) others non-polar constituents: Fatty acids: 1-Monobutyrin (2.10%) and Coumarin: 4, 6-dimethyl-2-benzopyrone (4.08%). GC-MS of the pet ether extract of galls *P. integerrima* (SUPYA-PC-PE) also showed presence of number of low polar volatile compounds mainly terpenoids, vitamins and fatty acids. Total 46 compounds were identified out of these 25 compounds having amount % age more than 1% are Terpenoids: 3-Carene (4.61%), (1R)-(+) Alpha-Pinene (4.36%), (1/-)-Camphene (2.75%), Beta-Phellandrene (6.74%), (1S)-(1)-Beta-Pinene (2.28%), Gamma-Terpinene (7.40%), O-Cymene (8.00%), Terpinolene (3.19%), Alpha-Terpineol (2.35%), (+)-Fenchol (1.05%), Pino Carveol (1.90%), Camphol (2.01%), (-)-Terpinen-4-ol (7.48%), (-)-Alpha-Terpineol (6.12%), Verbenone (1.54%), L-
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Bornyl Acetate (1.40%), Beta-Caryophyllene (2.49%), Spathulenol (1.58%), (-)-Beta-Caryophyllene epoxide (1.10%) and Beta-Eudesmol (1.04%). Others Non-polar constituents: Fatty acids: Octanoic acid (2.26%) and Myristic acid (5.91%), Vitamin: L-Ascorbic acid dihexadecanoate (3.70%) and Hydrocarbon Alkanes: Hexane 2, 4-Dimethyl (4.00%) and N-Tetratriacontane (1.07%).

Pharmacological Evaluation was initiated with the safety evaluation by Acute Toxicity Studies using Class method (Limit Test) as per OECD guidelines (423 a) both the drugs i.e. rhizomes of *H. spicatum* and galls *P. integerrima* were found to be safe causing no significant change in behavioral signs, toxic manifestations and along with zero mortality at different doses i.e. pet ether extract 2000mg/Kg and 5000mg/Kg and hydro-ethanolic extract in dose 2000 mg/kg and 5000 mg/kg. For evaluation of antitussive activity the effect of drug treatments on citric acid induced coughing in guinea pig was evaluated using different plant extracts. Pet ether extract 400 mg/Kg *(p.o.)* of both selected plants showed better antitussive effect with marked increase in cough latency and decrease in cough frequencies better than hydro-ethanolic extract 400 mg/Kg *(p.o.)* and comparable to standard codeine. Anti-asthmatic activity was evaluated using OVA induced asthma model in guinea pig for pet ether extracts of rhizomes of *H. spicatum* and galls of *P. integerrima* in comparison to aminophylline. Behavioral assessments of airway hyper responsiveness showed that the pet ether extract 400 mg/Kg *(p.o.)* of both selected drugs exhibited significant increase in cough latency time and decrease in cough frequency. Both didn’t even show any abnormalities with any signs of dyspnea. Pet ether extract 400 mg/Kg *(p.o.)* of rhizomes of *H. spicatum* exhibited comparable effect to aminophylline treated group (AMN). Cellular infiltration assessment for airway inflammation in terms of comparison of cellular concentrations in BAL fluid estimated by total leukocyte count (TLC) and eosinophilic count showed that pet extract 400 mg/Kg *(p.o.)* of both selected drugs exhibited significant effect comparable to aminophylline. Biochemical assessment for on airway inflammation based on effect of on lipid peroxidation, inhibition of OVA induced NOx and effect on glutathione concentration showed that pet ether extract 400 mg/Kg *(p.o.)* of both selected drugs exhibited significant effect comparable to aminophylline. Further the Lung histology revealed histopathological alteration where pet ether extracts 400 mg/Kg *(p.o.)* of both selected drug histological sections of lung tissue showed widening of
intra-alveolar septa because of vasodilation, edema and increased in macrophages comparatively equivalent to standard drug treated group (AMN). *In-vitro* anti-oxidant assay showed that standard antioxidant drug (ascorbic acid) has maximum anti-oxidant evident by free radical scavenging effect in terms of IC$_{50}$ Value. The volatile oils and pet ether extracts exhibited a concentration dependent free radical scavenging effect in terms of IC$_{50}$ values where pet ether extracts have better anti-oxidant potential as compared volatile oils. *In-vitro* anti-inflammatory assay showed that among volatile oils and pet ether extracts, pet ether extracts have better nitric oxide scavenging effect as comparable volatile oils. *In-vitro* immunomodulatory assay through cell viability assay using % proliferation in Human PBMC cells as indictor showed that volatile oil of *H. spicatum* exhibited immuno-suppressive at low concentrations and immuno-stimulatory activity at high concentrations which is evident by increased % age proliferation whereas pet ether of *H. spicatum* at lower concentration behaves as immuno-suppressive at all concentrations. Both volatile oil and pet ether of *P. integerrima* at lower concentration behave as immuno-suppressive but at high concentration behaves as immuno-stimulatory. *In-vitro* finding also indicated anti-oxidant and anti-inflammatory potential of pet ether extracts is comparatively more than volatile which further support additive effect of other volatile and non-polar constituents apart from terpenoids. All the finding for pharmacological activities strongly supports the role of non-polar and volatile constituents of pet ether extracts of both rhizomes of *H. spicatum* and galls *P. integerrima* with inherit considerable potential to be used in pulmonary disorders as fixed by the nature. The basis of pharmacological activities is hidden in the chemical composition of non-polar and volatile constituents in pet ether extracts and volatile oil. With the GC–MS, chromatographic fingerprint of the essential oil and pet ether extracts of these selected plants was developed, also the information related to its most qualitative and relative quantitative composition was revealed to represent the phytoequivalence. Major non-polar and volatile constituents more than 1% which showed the presence in pet ether extracts of rhizomes of *H. spicatum* were terpenoids, coumarins, and fatty acids. They have their bioactivity reported as anti-tussive, anti-asthmatic, anti-inflammatory, anti-oxidant and immunomodulator in the present study. The pet ether extract of galls *P. integerrima* also showed presence major non-polar and volatile compounds more than 1% namely terpenoids, vitamins and fatty
acids. The GC–MS of both the plants also revealed presence of various minor phytoconstituents having concentration less than 1% may also have contributed towards the overall pharmacological effect which cannot be ignored. The synergism occurring between the volatile and non-polar constituents cannot be overruled that may also have contributed towards the overall pharmacological effect. These plants were found to be potent anti-tussive, anti-asthmatic, anti-inflammatory, anti-oxidant and immunomodulators. These exhibited improvement in symptoms as well as pulmonary functions in mild to moderate asthma as indicated by anti-tussive potential, anti-asthmatic efficacy and better improvement in pulmonary functions. The anti-asthmatic property attributed to the presence of active constituents i.e. non-polar and volatile constituents of these plants as evidenced from GC-MS fingerprinting and interpretation.

**Conclusion**

On the basis of findings from present study it can be concluded that the rhizome of *H. spicatum* and galls of *P. integerrima* have potent anti-tussive and anti-asthmatic activities possible by anti-inflammatory, anti-oxidant and immunomodulatory mechanisms. These effects may be due to the presence of major non-polar volatile constituents, and hence these plants may be recommended to treat asthmatic disorders. The present study rationalizes the use of the non-polar and volatile constituents of rhizomes of *H. spicatum* and galls *P. integerrima* plants as anti-asthmatic agents.