GENERAL DISCUSSION

*Scoparia dulcis* L. is a distinguished folk medicinal plant and amongst the indigenous communities it has been broadly practised for its diverse pharmacological properties. Literature survey reflects that *Scoparia dulcis* L. possesses a wide range of phytoconstituents belonging to different groups of secondary metabolites. The experimentations on crude extracts as well as isolated metabolites to investigate the potential pharmacological possessions had documented its wide potentialities to exhibit analgesic, anti-inflammatory, antidiabetic, antioxidant, antiviral, antitumor, cytotoxic and hepatoprotective activities evaluated in both *in vivo* and *in vitro*. Although many researches have already been carried out in different parts of the world, very little work has been documented from North Eastern region of India, especially from Assam. Again, no work related to computational screening of the compounds from this plant has been reported yet. Hence, the present state of work was an attempt to investigate the phytochemical screening of *Scoparia dulcis* L. from Southern Assam as well as *in silico* evaluation of the potential bioactivity of the isolated metabolite(s).

The phytochemical screening with different crude extracts viz., Petroleum ether, Ethyl acetate, Acetone and Methanol confirmed the presence of alkaloid, saponin, tannin and flavonoid. These findings are supported by previously documented works from Nigeria (Yisa, 2009; Okhale *et al.*, 2010), Kerala (Muthumani *et al.*, 2010), Odisha (Mishra *et al.*, 2012), Zarkhand (Parekh *et al.*, 2011), Ebiraland (Ali *et al.*, 2011) and Southern Assam (Saikia *et al.*, 2013). Occurrence of these secondary metabolites had already been validated to possess various pharmacological properties, such as, presence of alkaloids had been reported to have anticancer, antidiabetic, anti-aging and antiviral properties (Evans & Trease, 2002), saponin had been reported to be cardio-tonics
(Evans & Trease, 2002), presence of tannin may be responsible for curing diabetes, diarrhoea, sore throat, skin ulcer and dysentery, while, flavonoids had been reported as a remedy to cancer, inflammations and allergies (Evans & Trease, 2002; Cushine & Lamb, 2005). Thus, these findings evidently provided essential information for chemical identification of this plant to initiate every possible effort for the discovery of new and varied pharmaceuticals.

The TLC profiling of the crude extracts of Petroleum ether, Ethyl acetate, Acetone and Methanol at different polarity ratios indicated the presence of diverse number of phytochemicals. Ethyl acetate and Acetone extracts revealed the presence of greater number of phytoconstituents compared to Petroleum ether and Methanol extracts. This result reflected an indication about the polarity of the components present as well as provided useful information regarding selection of the appropriate solvent system for the separation of the phytochemicals.

Regarding isolation of bioactive compound(s), by employing Column chromatography, a TLC grade pure fraction was isolated from Ethyl acetate extract of *Scoparia dulcis* L., using Petroleum ether and Ethyl acetate (9:1) as eluent. The fraction was further purified in pHPLC and the pure compound was isolated. After analyses and interpretation of different spectroscopic data generated by Infrared spectroscopy (IR), Nuclear Magnetic Resonance spectroscopy (NMR) and Mass spectrometry (MS), the structure of the compound was elucidated and found to be (E)-7-methyl-2-(5,6,7,8-tetrahydronaphthalen-2-yl)oct-5-en-3-one, a ketonic compound. **Literature survey revealed that the compound has not been reported previously from Scoparia dulcis L. or from any other plant species i.e., this piece of work led to the isolation of a novel compound.** It may be worth mentioning that till date 71 compounds have been reported from *Scoparia dulcis* L. Structurally and phytochemically the compound (E)-
7-methyl-2-(5,6,7,8-tetrahydronaphthalen-2-yl)oct-5-en-3-one, isolated from the present work does not match with any of these. As such, may be concluded as an additional report from the plant. The nature of the compound was white, amorphous and soluble in organic solvent.

Nowadays, Computer aided drug design is a very promising tool for designing and optimizing new, effective and safe lead molecule that can also reduce the time as well as expenses associated with the process of drug discovery. Hence, before proceeding to in vitro and in vivo approaches, the in silico screening of the compound is ideal for predicting probable bioactivity, evaluating the actual mechanism of action and other pharmacological parameters which could be beneficial in designing a lead molecule for a definite therapeutic utility. Hence, to begin with, the compound isolated from Scoparia dulcis L. was checked in some existing databases for any previous records and the result indicated that the compound has never been reported earlier depicting the novelty of the compound.

The drug likeness and Lipinski filter screening of the compound satisfied all the parameters and obtained the drug likeness model score of 1.17. This indicates that the newly isolated compound possesses all the properties to become a drug like molecule. Again, the ADME/Tox screening which defines the disposition of a pharmaceutical compound within an organism, exhibited no toxicity risk and the compound satisfied all the parameters i.e., the compound is nontoxic. After confirming non-toxicity of the compound, the target fishing of the compound (E)-7-methyl-2-(5,6,7,8-tetrahydronaphthalen-2-yl)oct-5-en-3-one, to predict the probable drug target revealed the highest potentiality to inhibit 2-Hydroxy-6-Oxo-6-Phenylhexa-2,4-Dienoate Hydrolase BPHD (HsaD) enzyme of Mycobacterium tuberculosis H37Rv strain. Although 71 compounds have been reported from Scoparia dulcis L., many of them are
reported to possess cytotoxic, antitumor, antidiabetic and antiviral activities but, none of these have been reported with antitubercular activity. Since, this compound exhibited antitubercular activity \textit{in silico}, the current study has opened up a new possibility of usefulness of the plant for a very significant disease like tuberculosis.

\textit{Mycobacterium tuberculosis} infection is considered as one of the leading cause of deaths worldwide. An estimated 2 million people annually and approximately 2 billion of world’s population are severely infected with this organism. It is an intracellular pathogen which resides in a modified phagosome of the host macrophages and has adapted unique ways of thriving in this harsh environment (Miner et al., 2009). The mechanism underlying the remarkable ability of this pathogen to survive for longer periods within the host is poorly understood. Recent studies demonstrate that the pathogenic strain utilizes cholesterol as a growth substrate for its survival within the host environment. Highlighting the importance of cholesterol in bacterial pathogenesis, the deletion of genes involved in cholesterol metabolism reduces the virulence of \textit{Mycobacterium tuberculosis} (Lack et al., 2010). The cholesterol metabolism operon of \textit{Mycobacterium tuberculosis} has been identified and includes the HsaA-D genes. Gene deletion mutants of HsaC and HsaD have shown that these enzymes are required for survival of the pathogen inside macrophage. HsaD is a member of meta-cleavage product (MCP) hydrolase class of enzymes, which catalyses the cleavage of 4, 9-DHSA within the cholesterol metabolism pathway. It cleaves carbon-carbon bonds \textit{via} a serine protease like catalytic triad. As HsaD is an essential gene for cholesterol metabolism and its catalytic pathway helps in growth of the pathogen inside the host macrophage, it can be a promising target for antitubercular therapy (Ryan et al., 2014).

Traditional report is available on \textit{Scoparia dulcis} L. to cure pulmonary tuberculosis by the tribal people in West Africa (Benin) (Adjanohoun et al., 1989).
Although no further record is found regarding antitubercular activity of this plant, some other member of the family Scrophulariaceae are known to possess antitubercular activity. *Herbscum thapsus* which is commonly known as Mullein has been reported as a common remedy of tuberculosis (Turker and Camper, 2002). Again, several terpenes isolated from *Calceolaria pinnifolia* Cav. were also evaluated for their antimycobacterial activity (Woldemichael *et al.*, 2003). Literature review also revealed that *Scoparia dulcis* L. possesses significant antimicrobial activity and some of the compounds isolated from this plant *viz.*, 4-epi-scopadulcic acid B, dulciol, isoludcinol and scopadulcic acid C had been experimented against *E. coli*, *B. subtilis* and *S. aureus* (Phan *et al.*, 2006). Again, Latha *et al.* reported various concentrations of chloroform and methanol fractions against different bacterial strain exhibited significant antimicrobial activity compared to respective reference drugs (Latha *et al.*, 2006). Report on antimicrobial activity of this plant is also available that has been tested with different crude extracts against *E. coli* and *Staphylococcus* sp. (Saikia *et al.*, 2012). But until now, no active principle has been reported so far from this ethnomedicinal plant with antitubercular activity. Since, the novel compound (E)-7-methyl-2-(5,6,7,8-tetrahydronaphthalen-2-yl)oct-5-en-3-one, isolated from *Scoparia dulcis* L. showed potential to inhibit HsaD enzyme of *Mycobacterium tuberculosis* H37Rv strain, which is a target for tuberculosis, the compound was further screened to evaluate the actual mechanism of action and to predict other pharmacological parameters which may lead to the discovery of a potent antitubercular agent.

Therefore, to evaluate the mechanism of action of the compound (E)-7-methyl-2-(5,6,7,8-tetrahydronaphthalen-2-yl)oct-5-en-3-one and the target HsaD enzyme, an array of *in silico* analyses were performed. The molecular docking study to evaluate the binding efficacy of the compound (E)-7-methyl-2-(5,6,7,8-tetrahydronaphthalen-2-
yl)oct-5-en-3-one with the target HsaD enzyme of Mycobacterium tuberculosis H37Rv strain revealed good binding affinity with the active site of the enzyme. To compare the findings, 23 standard inhibitors were also docked in the same active site of the target and the result indicated that the compound exhibited better binding affinity than 10 out of 23 standard inhibitors. On studying the interaction patterns, it was observed that the ketonic oxygen atom of the compound was mainly responsible for the formation of Hydrogen bonds with the target. Also, by studying the interaction patterns of 23 standard inhibitors specified that the amino acid H GLY-45-B was found to be the crucial amino acid for the formation maximum hydrogen bonds which also justified the findings.

To study the correlation of structural or property descriptors of compounds with activities Quantitative Structure Activity Relationship (QSAR) study was performed. QSAR is applied in many disciplines including drug design and environmental risk assessment. Since it is based on activity data, QSAR has the advantage of modelling in vivo situation (Azhar et al., 2006). For QSAR analysis, Molecular mechanics based and Density functional theory (DFT) based descriptors for all the 23 standard inhibitors were considered along with their respective IC$_{50}$ values. The multiple linear regression analysis was performed using these descriptors as independent variable and activity log(IC$_{50}$)$^{-1}$ as dependent variable. The analysis provided the R$^2$ value of 81.79% and the adjusted R$^2$ value of 79.97%. The higher R$^2$ value indicates greater fitting of the equation with the dataset and also the higher significance of the regression analysis. From the regression analysis, the QSAR equation was generated and by putting the descriptor values for the isolated compound (E)-7-methyl-2-(5,6,7,8-tetrahydronaphthalen-2-yl)oct-5-en-3-one, the activity (IC$_{50}$) of the compound was predicted and found to be 2.570 nM which is better than all the 23 standard inhibitors.
The scattered plot against experimental and predicted activities also displayed good prediction by the QSAR equation, which validated the QSAR model.

Thus, this piece of work on phytochemical screening and *in silico* therapeutic evaluation of *Scoparia dulcis* L. led to the isolation of a novel metabolite (E)-7-methyl-2-(5,6,7,8-tetrahydronaphthalen-2-yl)oct-5-en-3-one. Since, the isolated compound showed activity against HsaD enzyme of *Mycobacterium tuberculosis* H37Rv strain, which is a very recently identified and promising target for tuberculosis (Ryan *et al.*, 2014) and no standard drug is available yet in the market, this may be assumed that the compound (E)-7-methyl-2-(5,6,7,8-tetrahydronaphthalen-2-yl)oct-5-en-3-one may possess the potentiality to become a lead molecule for treating tuberculosis. However, this piece of endeavour demands further exploration in *in vivo* model to validate the findings of *in silico* analyses.