2.1 **RESEARCH ENVISAGED:**

The plants have been in use for their medicinal values since ages and have also been explored by the researchers to discover the active phytoconstituents responsible for their biological effect (Kyriakopoulou et al., 2013). The different parts of same plant may contain different constituents and may be associated with different biological effects as well. Several studies have been reported on different parts of plants and phytoconstituents responsible for their effect *viz.* leaves of *Azadirachta indica* contains the nimbin and nimbanene (Baraldi et al., 2008), fruit peel of *Punica granatum* contains ursolic acid (Hossain et al., 2013), seeds of *Syzygium cumini* contains ellagic acid, isoquercetin (Mohammad and Madkashani 2012), the bark of *Cinnamomum zeylanicum* contains procyanidins and catechins (Ayyanar and Pandurangan, 2012), flowers of *Jasminum sambac* contains coumarins (Rao and Gan, 2014) etc. These phytoconstituents may serve as biomarkers for particular biological effect and estimated in terms of their quantity and quality using different methods of estimations like HPTLC, HPLC, LC-MS, GC-MS etc. (Kunhanchan et al., 2012; Xie et al., 2006; Pozharitskaya et al., 2007; Pirisi et al., 2000; Deferera et al., 2000).

Different methods of extractions may cause different extent of extraction for different phytoconstituents. Extraction of plant material had been the traditional method to get the active principles from plant materials and the most common techniques of extraction were room temperature extraction, maceration, Soxhlet extraction and decoction. Now, recently many researchers have reported the novel methods of extractions like the MAE (Microwave assisted extraction), USAE (Ultrasound assisted extraction), SFE (Supercritical fluid extraction) (de la Torre-Carbot et al., 2005; Nunez Selleys et al., 2002; Huddleston et al., 1998; Hawthorne et al., 2000; Khan et al., 2010; De Castro and Garcia-Ayuso, 1998; Pourmortazavi and Hajimirsadeghi, 2007). These novel methods have certain advantages over...
the classical methods of extractions like the better extraction yield, better yield of the marker compounds, better biological response and less extraction time.

Among the novel techniques of extraction, the ultrasound assisted extraction works on the phenomenon of penetration of ultrasound waves in the cells of plant materials and causing vibrational changes leading to enhanced cell permeation and better extraction (Dai and Mumper, 2010). Many studies have been reported for the USAE describing its synergistic effects to carry out better extraction of bioactive principles (Saleh et al., 2006; Singanusong et al., 2015; Herrera and De Castro, 2005; Kimbaris et al., 2006; Carrera et al., 2012). Another technique, the microwave assisted extraction has also gained importance due to its oscillating perpendicular magnetic and electronic waves causing more penetration of solvent inside the plant cells and better extraction. The microwave assisted extraction and the ultrasound assisted extraction depends upon certain factors such as the irradiation time, power, temperature range, solid to solvent ratio and type of solvent that contributes to variable results. These parameters have to be optimized using the design of experiments to select their values to carry out the experiments (Kaufmann et al., 2002, Lu et al., 2008, Routray and Orsat, 2012).

The *Terminalia arjuna* has also been reported to be associated with various biological effects like antiulcer, anti-inflammatory, hypolipidemic, cardiotonic, wound healing, anti-diabetic, antioxidant activity and immunomodulatory etc. (Duthie et al., 2016; de Beer et al., 2016; Elufioye and Onoja 2016).

- Further, the *Terminalia arjuna* has very good antioxidant effect which is due to the presence of the phenolic acids as reported in the literature (Sultana et al., 2007). The arjunic acid and arjunolic acid have been reported as major biomarker antioxidant compounds present in *Terminalia arjuna* stem bark (Manna et al., 2008, Sun et al., 2008, Elsherbiny et al., 2016, Sumittra et al., 2001). As antioxidant effect of *Terminalia arjuna* can help to treat various disorders associated with the free radical production so, to enhance the yield of biomarker compounds...
arjunic acid and arjunolic acid, different novel extraction techniques need to be applied.

- The in silico approach has led the researchers to find a tool to screen and find the inhibition sites of various enzymes promoting different body disorders and thus has been reported as well for the docking and inhibition studies by various phytoconstituents.

- The *Terminalia arjuna* has been reported as anti-diabetic in very few studies like it has been mentioned in the study reported by Ragavan and Krishnakumari, 2006, but the α-amylase inhibitory effect of TA has not been mentioned specifically. Hence, there is a strong need to evaluate the in silico and in vitro effect of *Terminalia arjuna* on α-amylase inhibition.

- The urease is a nickel containing metallo-enzyme and inhibition of the metal site leads to inactivation of enzyme. The *Terminalia arjuna* has nickel inhibitory effect as suggested in the study reported by Rajput et al., 2015. Hence, there is a need to test the urease inhibition potential of *Terminalia arjuna* using in-silico and in vitro methods.

- The effect of *Terminalia arjuna* has been mentioned as effective antibacterial specially for Gram negative strains while only few studies have been reported for its antifungal effect, hence, it was planned to explore the effect of novel extraction techniques on the antimicrobial efficiency of this plant in this study.

- Glutathione is the most readily available nucleophile, which can attack any free-radical responsible for oxidative stress and does not need any activation. The decreased levels of GSH also contributed to severe hypertension via production of oxidative stress. The decrease in levels of GSH can be attained by administration of GSH synthase inhibitors such as buthionine sulfoxamine (BSO) to produce the oxidative stress and hypertension in animal models (Vaziri et al., 2000; Ganafa et al., 2002; Bayorh et al., 2003; Rodriguez-Gomez et al., 2010; Banday et al., 2007; Allam et al., 2013; Manning et al., 2005; Ganafa et al., 2002).
The *Terminalia arjuna* plant has also been associated with the cardioactive principles *viz.* cardio-protective, antihyperlidemic, cardiotonic, hypocholsteremic, anticoagulant, antithrombotic (Bharani et al., 1995; Dwivedi et al., 1989; Chaudhari et al., 2007; Singh et al., 2008; Jain et al., 2009 and Halder et al., 2009). As seen from the literature the clinical use of *Terminalia arjuna* was in practice since ancient times while a few scientific studies have been reported for anti-hypertensive effect of *Terminalia arjuna* (Colabawalla, 1951). In a study, the *Terminalia arjuna* bark (*Arjun chhal*) was tested on the patients for congestive heart failure and hypertension clinically and 500mg/kg body weight was found to be the effective dose for patients of congestive heart failure, another study described the dose dependent hypotensive effect in anaesthetized dogs by aqueous and alcoholic extract of *Terminalia arjuna* bark, similar study on anesthetized dogs suggested for the dose dependent hypotensive effect of 70% ethanolic extract of *Terminalia arjuna* on dog models (Verma et al., 1998; Dwivedi and Chopra, 2014; Yegnanarayan et al., 1997; Nammi et al., 2003; Takahashi et al., 1997). However, the exact mechanism of the antihypertensive effect of *Terminalia arjuna* is still not very clear and the biomarkers compounds responsible for the antihypertensive effect have also not yet been conclusively established.

The free radical mainly oxidizes the biomolecules present at different sites and creates problems. In order to overcome these problems the antioxidant compounds cause deactivation of free radicals by donation of hydrogen atoms (Prasad and Kalra, 1993; Puthur, 2016). The toxic and carcinogenic effects of the currently used antioxidant preservatives like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have made the researchers to find better antioxidants. Further, several plants including *Terminalia arjuna* have also been reported for their prominent antioxidant effects and have fostered the choice of researchers towards them (Galano et al., 2016; Fang et al., 2016; Choi et al., 2016; Choi et al., 2016; Reddy and Grace, 2016; Ogbole et al., 2016).
Diabetes is a disorder related to elevated blood glucose levels in the body and the same has been associated with complications in the body making an individual more prone to cardiac and other problems thereof. The \( \alpha \)-amyrase enzyme has been involved in the metabolism of hydrolyzed starch and glycogen molecules yielding the glucose and maltose, causing hyperglycemia and developing the type 2 diabetes as such (Shankaraiah and Reddy, 2011). Different researchers have designed different synthetic and semi synthetic drugs to inhibit this enzyme (Tamil et al., 2010; Jyothi et al., 2013; Kim et al., 1999; Wei et al., 2014 and Xu et al., 2014, Kumar et al., 2013).

The existing inhibitory drugs including the standard acarbose used for oral hypoglycemic effect have been reported for GIT side effects caused due to excessive pancreatic \( \alpha \)-amyrase inhibition (Hussain et al., 2012; Hamdan et al., 2004; Verspohl, 2012 and Mogale et al., 2011). Further, the \( \alpha \)-amyrase inhibitors derived from plants have been reported to show lesser inhibitory effect and again encouraged the researchers to search for a better plant material for this bioactivity (Bale and Gavade, 2014; Saha and Verma, 2012).

The urease is supposed to be a virulence factor and is found in different pathogenic bacteria. It colonizes into the host organism and is involved in maintenance of cells of bacteria in the tissues. The urease enzyme has a toxic effect on human cells and the presence of its ureolytic activity may serve as an important marker for a number of bacterial infections. The urease has been reported as an immunogenic protein which is recognized with the antibodies present in sera of human beings which are associated with several long lasting disorders viz. atherosclerosis, rheumatoid arthritis or urinary tract infections (Konieczna et al., 2012). The acid acclimation that promotes the Helicobacter pylori to colonize in the acidic environment of GIT is also associated with the urease activity. The urease has also been reported to promote the bacterial and fungal infections in the body (Rutherford, 2014). The existing drugs causing the inhibition of urease including the thiourea have been associated with the side effects such as hypothyroidism, bradycardia, GIT disorders etc. (WHO, 2013). Several researchers have reported their work for urease inhibitory
effects of plant materials giving promising results (Ismail et al., 2016; Shaikh et al., 2015; Romero et al., 2015).

The problem of microbial spoilage and infections had been a challenge for the researchers since years but the problem of resistance has enhanced this challenge multifold causing autoimmune disorders (Jones et al., 2008, Cohen 2000, Cahill et al., 1997, Tysk et al., 1998). Different existing drugs having antimicrobial effects have been associated with the serious side effects which led the researchers to search for better alternatives. Several researchers have reported different plant materials anion, an oxygen-derived free radical. Hence, the superoxide anion can act as vasoconstrictor and is responsible for nitric oxide (NO) biosynthesis and bioavailability (Dikalov et al., 2016). Further, the decrease in nitric oxide levels is associated with the increase in oxidative stress and hypertension. Different models for evaluation of antihypertensive effects of drugs in animals have also been reported, viz., the genetic hypertension model induced via addition of extra sodium chloride in diet of the rats or through the use of transgenic hypertensive rats, endocrine hypertensive rat model induced via DOCA and high salt intake, stress-induced hypertension induced via loud noise, flashing lights, cold or hot stimuli, renal hypertension induced through two-kidney one-clip, one-kidney one-clip or two-kidney two-clip arrangements, oxidative stress induced hypertension via GSH synthase inhibitors, nitric oxide synthase inhibitors etc. (Gong et al., 2015; Ribeiro et al., 2015; Xiao et al., 2015; Padmanabhan et al., 2015; Emre et al., 2015; Dobrian et al., 2001; Ribeiro et al., 1992).

In view of the immense therapeutic potential of Terminalia arjuna there exists a strong need to enhance the yield of biomarker compounds and to improve the extraction efficiency of Terminalia arjuna using novel methods of extraction, to evaluate the extracts for their biomarker yield and their in silico, in vitro as well as the in vivo biological effects. Several researchers including our team had reported for the extraction and estimation of different bioactive plant material using microwave assisted extraction and ultrasound assisted extraction techniques (Devgun et al., 2010, Devgun et al., 2012, Devgun et al., 2013, Spingo and De Faveri, 2009).
In light of abovementioned facts, the present study was designed in accordance with the following objective and plan of work:

2.2 Objective of the study:

Effect of novel extraction methods, biological studies and evaluation of *Terminalia arjuna* bark.

2.3 Plan of work:

A. Collection and Authentication of Plant Material
B. Preparation and Evaluation of Conventional Extracts
   I. Room Temperature Extraction
   II. Soxhlet Extraction
C. Preparation and Evaluation of Novel Extracts
   I. Ultrasound Assisted Extraction
   II. Microwave Assisted Extraction
D. Optimization of Parameters for Novel Extracts
E. Biological Evaluation of Conventional and Novel Extracts
   I. Free Radical Scavenging Activity
   II. α-amylase Inhibitory Activity
   III. Urease Inhibitory Activity
   IV. Antimicrobial Activity
   V. Antihypertensive Activity
F. Compilation of Results and Interpretation of Data
G. Publication of Dissertation Report