

### **1.2.7 Literature on clinical study of puerperal bacterial infection.**

WHO [82] defines puerperal sepsis as an infection of the genital tract occurring at any time between the onset of the rupture of membranes or labour and the 42<sup>nd</sup> day postpartum in which fever and one or more of the following are present: pelvic pain, abnormal vaginal discharge, abnormal odor or discharge, and delay in the rate of reduction of size of the uterus.

Over the past few decades there has been a dramatic increase in the number of puerperal infected patients. Puerperal sepsis is responsible for 5% to 23% of all maternal deaths worldwide, and causes the loss of more than one third of woman's healthy years of life because of resulting illness [82-85]. In developing countries, puerperal sepsis causes at least 75,000 maternal deaths every year [78]. In Africa, it was estimated that 9.7% (95% CI 6.3-12.6) of maternal deaths were due to puerperal sepsis [86]. It has not only found puerperal sepsis in developing countries, but also in western countries, USA, and Japan [87-92]. Gram-positive cocci aerobic bacteria such as *Staphylococcus aureus* and *Staphylococcus epimermidis*, Group A, B streptococcus were the most commonly species found in puerpeal infections in Japanese reports, infection with the fungi, *Candida albicans*, was also found (in percentage of 51, and 2.6, respectively) [91].

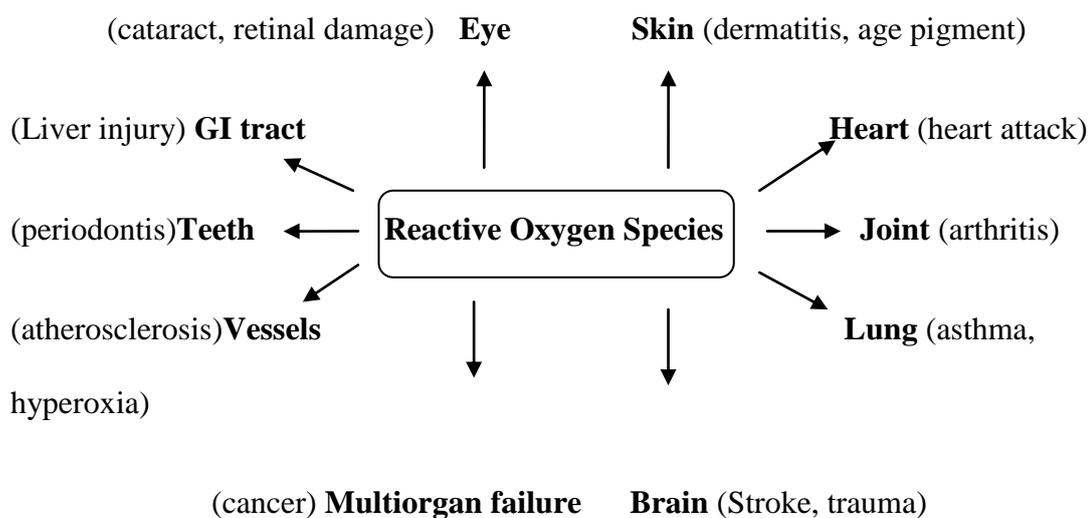
Data from the literature review also found that the various native plants were recorded for treating infectious diseases in puerperium in many ethnic groups in the world [93-94]. However, there has not been a clinical report on the effectiveness of postpartum herbal bath shown in the literature.

### 1.2.8 Free radical, oxidative stress and antioxidant activities

Free radicals are highly reactive atoms or molecular species that contains one or more unpaired electrons in its outer orbital [95-96]. They can not be alone, so they easily form a new bond with both radical and non-radical molecules via a variety of reactions including: the donation of the free radical's unpaired electron to a non-radical molecule or the formation of a pair with an electron from the other molecule by as an electron receiver, these free radicals include the hydroxyl radical ( $\text{HO}^\bullet$ ), superoxide anion ( $\text{O}_2^{\bullet-}$ ), nitric oxide ( $\text{NO}^\bullet$ ), and peroxy radical ( $\text{RO}_2^\bullet$ ) [96]. A reactive oxygen species (ROS) is defined as free radical or non-radical species which can cause a free radical reaction, examples of these are peroxylnitrite ( $\text{HNOO}$ ), hypochlorous acid ( $\text{HOCl}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), singlet oxygen ( $1\Delta\text{gO}_2$ ,  $1\Sigma\text{g}^+\text{O}_2$ ) and ozone ( $\text{O}_3$ ) [96].

Free radicals can be produced from endogenous sources such as the metabolism of both intracellular and extracellular reactions and stress whereas some species arise from exogenous sources such as radiation, smoking, other environmental causes [97-98].

Oxidative stress is the term referring to a serious imbalance between reactive species production and antioxidant defense that leads to cell and organ damage by the following mechanisms: breaking of carbohydrate, proteins, lipids, DNA molecules which can cause mutagenesis, protein inactivation, mitochondrial damage, receptor malfunction, and membrane disruption, these consequent diseases are the development of cancer, neurodegenerative disorders, cardiovascular diseases, and other diseases [99]. The biological damage of the body is shown in Figure 1 [100].



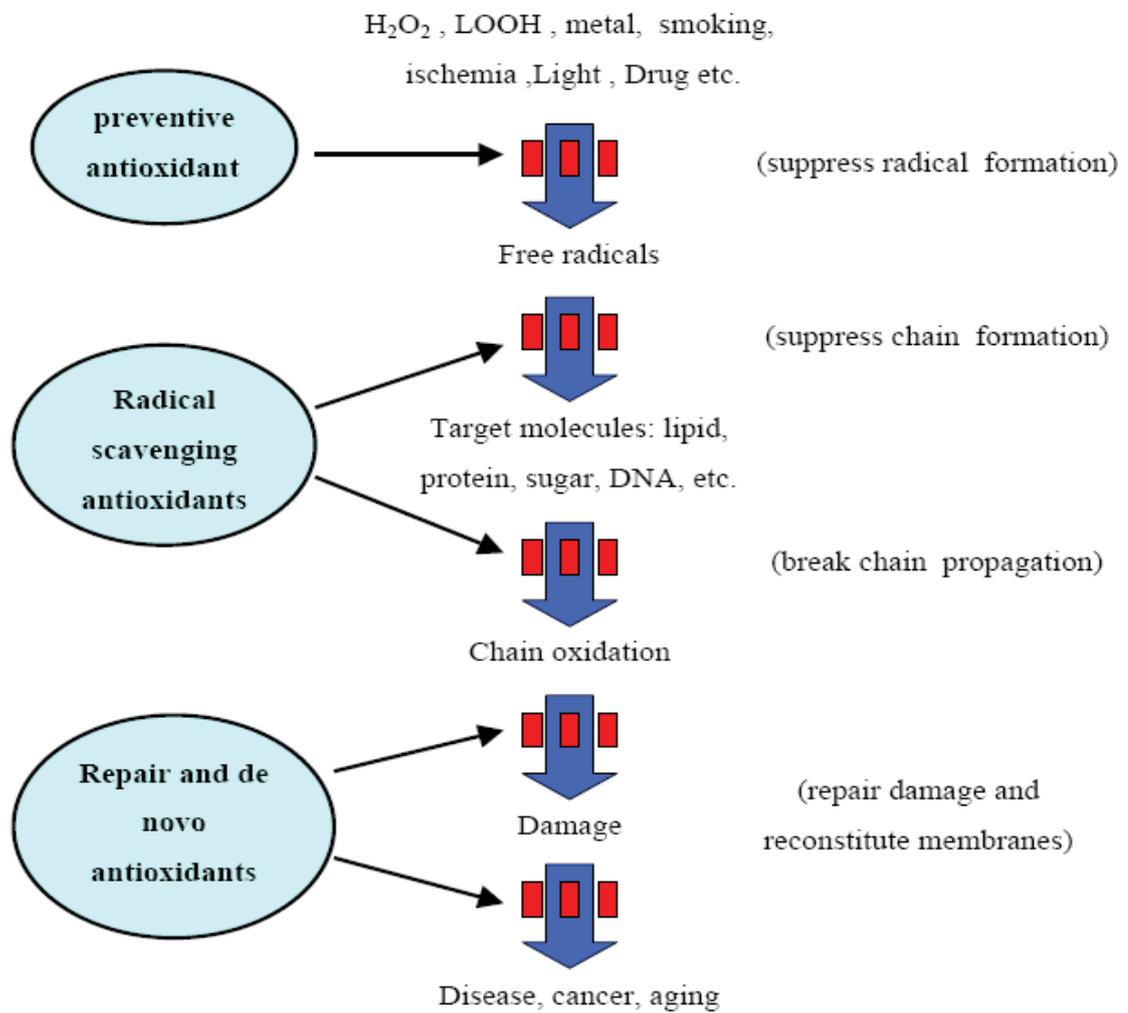
**Figure 1** Biological damage caused by ROS in human [100]

Antioxidants are any substances that can react with free radicals and result in cells and organ being protected from damage [101]. They can act at different levels including lipid peroxidation [102-103]:

1. Decrease localized  $O_2$  concentrations (e.g. sealing of food stuffs under nitrogen)
2. Prevent first-chain initiation by scavenging initiating radicals such as  $OH^\bullet$ .
3. Bind metal ions in a form that will not generate initiating species such as  $OH^\bullet$ , ferryl, or  $Fe^{3+}/Fe^{2+}/O_2$  and/or will not decompose lipid peroxides to peroxy or alkoxy radicals.
4. Decompose peroxide by converting it to non-radical products, such as alcohol.

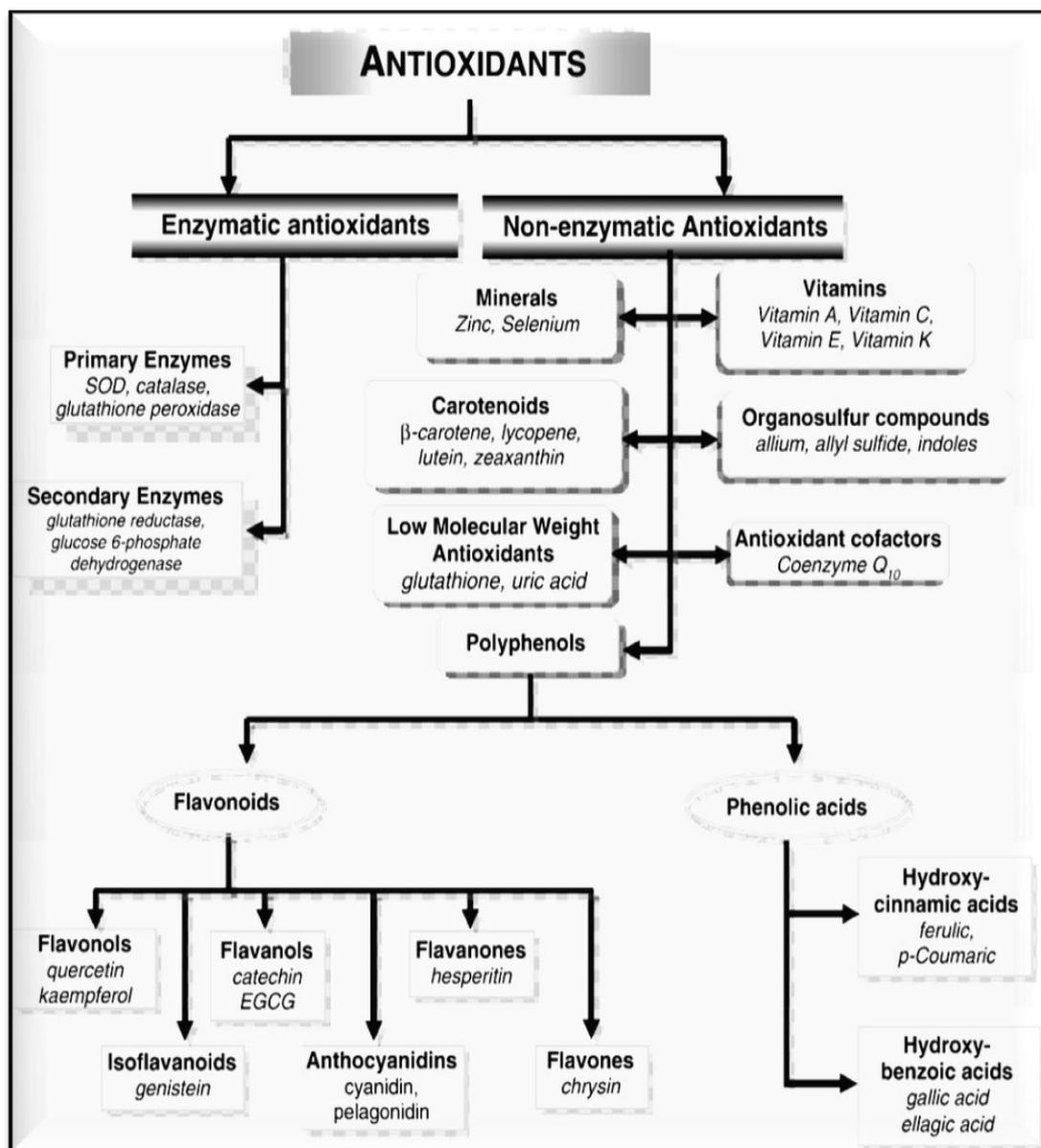
5. Chain-breaking, i.e. scavenging intermediate radicals such as peroxy and alkoxy to prevent continued hydrogen abstraction. Chain-breakers are often phenols and amines

Antioxidants acting by mechanism 1, 2, and 3 can be called “preventive antioxidants” while some substances that use mechanism 4 are also “preventive antioxidants”, whereas substances that use mechanism 5 are called “chain breaking antioxidants”. Antioxidants may act with multiple mechanisms as is shown in Figure 2 [104].



**Figure 2** Defense systems *in vivo* against oxidative damage [104]

Biological antioxidant sources can be produced from 2 sources: endogenous sources which are enzymatic antioxidants whereas exogenous sources are the non-enzymatic or dietary antioxidants [99, 105]. These antioxidants are shown in Figure 3.



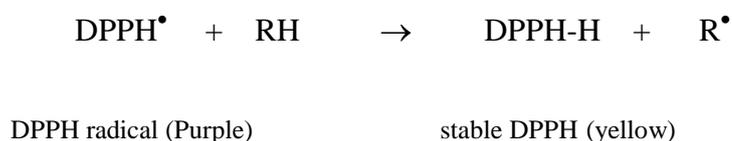
**Figure 3** Classification of antioxidants [99]

Various reactions are used to determine antioxidant activity [96-101] such as:

1. ABTS assay: ABTS (2,2'-azinobis-[3-ethylbenzothiazoline-6-sulphonic acid) is a peroxidase substrate which can be oxidized by peroxy radicals, metmyoglobin or other oxidants in the presence of hydrogen peroxide. The reaction

generates a metastable cation. ABTS<sup>•</sup> which is intensely colored can then be monitored spectrometrically at a range of 600-700 nm.

2. DPPH<sup>•</sup> radical scavenging activity assay: The DPPH<sup>•</sup> radical is a purple stable radical. In a assay, the purple chromogen radical is reduced by antioxidant/reducing compounds to a pale yellow. The reducing ability of antioxidants can be measured at 515-528 nm after 30 minutes of reaction. The free radical scavenging activity of DPPH can be shown as the following equation:

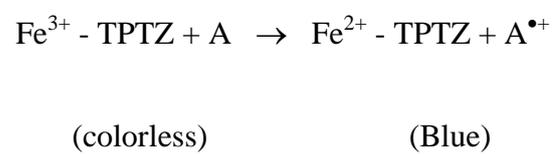


From the equation, plants extracts provide proton donors, and act as free radical scavengers.

3. Anti-lipid peroxidation assay: This assay can be performed by using  $\beta$ -carotene bleach via auto-oxidation, which is oxidation induced by light, heat, or by peroxy radicals. The decolorization can be diminished by antioxidants and then measured at 470 nm. Thiobarbituric Acid Reacting Substances (TBARS) assay is also used. Lipid peroxidation is produced by adding metal ions such as Fe<sup>2+</sup>, FeCl<sub>3</sub> and ascorbate. An antioxidant reaction can then be produced by a peroxy radical scavenging and metal ion chelation reaction.

4. Reducing power assay: This assay is called Ferric Reducing Antioxidant Power (FRAP) assay. This assay uses an oxidation/reduction reaction to measure the ability of a sample for reducing ferric iron (Fe<sup>3+</sup>) to ferrous iron (Fe<sup>2+</sup>). The reaction

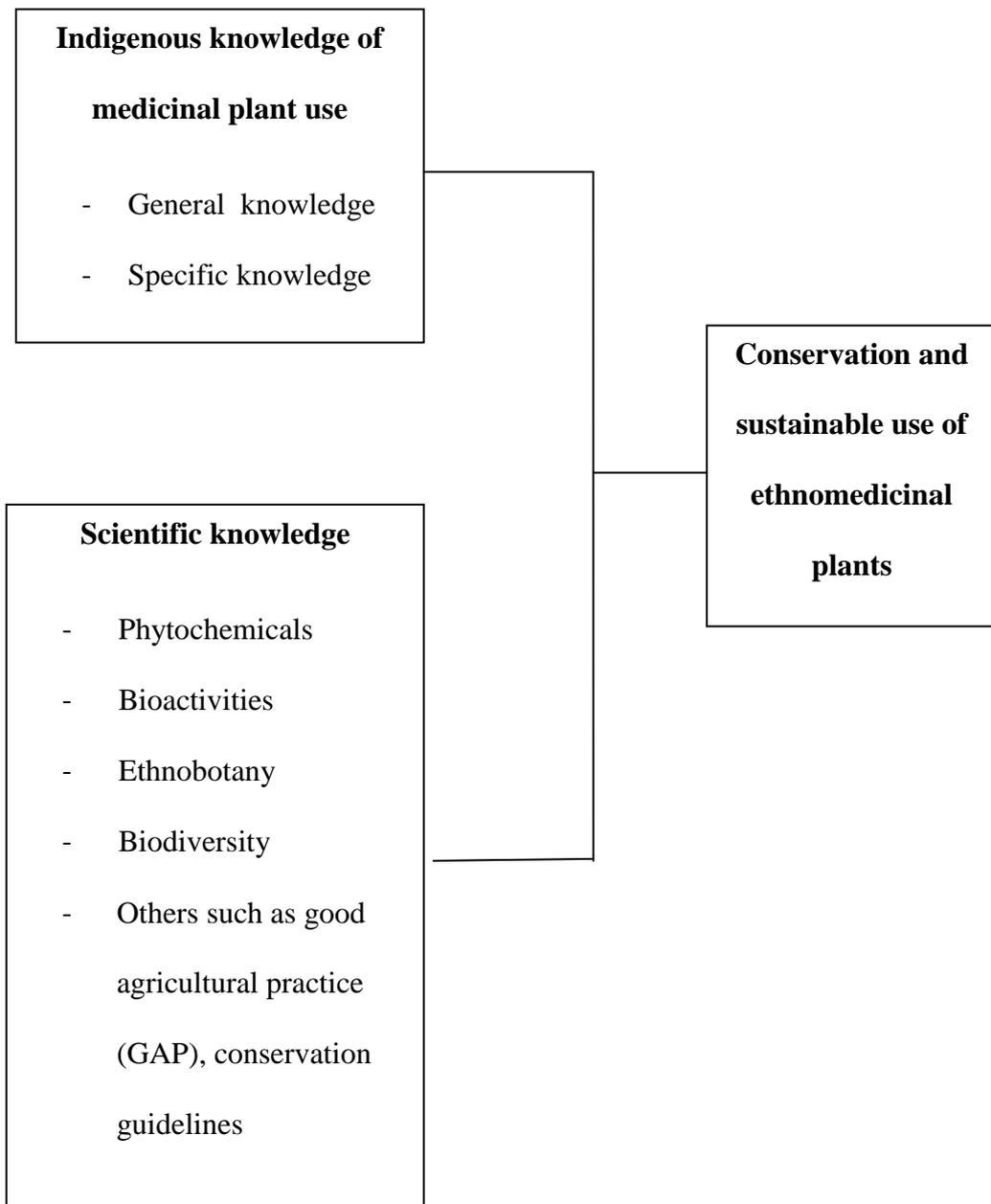
can then measure the appearance of blue color at 595 nm. The ferric reducing power can be shown as the following equation:



This research aims to study and record knowledge of the use of medicinal plants and medicinal plant resources in Sancharum Village to determine the current status of medicinal plants there. A report of this information was then disseminated to the community leaders for the establishment or revision of local policies on indigenous forestry for the protection of medicinal plant resources and biodiversity resources in the mountainous area of Nan Province. To confirm this traditional use, promote sustainable use, and to develop the local people's knowledge, the phytochemical and biological activities such as anti-oxidant and antimicrobial activity of interesting medicinal plants were tested.

### **1.3 Conceptual framework**

The conceptual framework of this study was indigenous knowledge of medicinal plant use can be conserved by manage indigenous knowledge directly and it also can be conserved by add scientific knowledge into indigenous knowledge to empower and strengthen this knowledge for sustainable use of medicinal plants in the Mien community as following chart:



#### **1.4 Research Objectives**

1. To conserve medicinal plants which have high potential value for primary healthcare of the Mien in Sancharurn village for sustainable use in their community.

2. To survey and record medicinal plants used by the Mien in Sancharurn Village to collect them as a inventory book that can be easily access this knowledge by the young Mien and this book can be used as reference book in community.

3. To determine the chemical compounds and biological activities of the medicinal plants most frequently used in traditional medicine of the Mien in Sancharurn village in order to validate the usefulness of the Mien's medicinal plants.

#### **1.5 Scope of the Research**

This study is scientific research (using an integrated methodology to collect both qualitative and quantitative data) that focuses on the use of medicinal plants by the Mien for postpartum treatment in Sancharurn Village, Pathong District, Nan Province leading to the conservation of medicinal plants and their habitat, and to promote sustainable use and development.

This research is divided into three parts. First, a survey of medicinal plant knowledge and habitat in Sancharurn Village, Nan Province, Thailand was conducted. Second, experimental research on the chemical compounds and bioactivity of selected plants was performed. Last, development of conservation and sustainable use of medicinal plants in community using participatory action research.

## 1.6 Operation definitions

**Conservation:** The smart use of plant resources at the rate that is most cost-effective. This conservation can be divided into two parts: conservation of knowledge regarding medicinal plants used, and conservation of medicinal plant diversity. The conservation of medicinal plant diversity can be divided into two parts: *in situ* conservation, which is the conservation of plants in their habitat such as the establishment of home and community gardens. *Ex situ* conservation is the conservation of plants outside of their habitat [106]. This research used applied *ex situ* conservation by did the research on phytochemicals present in the plant and bioactivity of selected medicinal plants to prove and strengthen knowledge of medicinal plants used by the Mien.

**Ethnomedicinal plant:** Medicinal plants (planted that cultivated and used for medicinal purposes) which are traditionally used by local people for their health care.

**Sustainable use:** the UN's definition [7-8]: "the use of components of biological diversity in a way and at a rate that does not lead to the long-term decline of biological diversity, thereby maintaining its potential to meet the needs and aspirations of present and future generations". This study emphasizes the sustainable use of medicinal plants of children by providing both knowledge and materials for reinforcement to the community and public school in the community.

**The Mien (Yao):** Ethnic people, who use a Sino-Tibetan language and are well known by their use of medicinal plants, migrated from Southwestern China and are now residing in 9 provinces of northern Thailand [10]. Sancharurn Village, Nan Province is the selected village for this study.